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Evaluation of the Effects of Extremely Low Frequency Electromagnetic Fields on Movement in the Marine Diatom *Amphora coffeaeformis*

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Abstract. Published work has shown that population motility in the marine diatom *Amphora coffeaeformis* can be influenced by externally applied electromagnetic fields (EMFs). Here we report attempts to repeat these experiments, which have been proposed as a model for assessing the effects of EMFs on biological systems. Susceptibility to EMFs was tested using five strains of diatoms on agar plates at a very broad range of field conditions, but no effect on population motility was demonstrated. Exposure period to the EMFs, cell density, and position in the cell cycle had no effect on EMF susceptibility, and the direction and distance moved by the diatoms were not affected by EMFs. When tested after at least a month of pre-incubation at 20 μT, diatoms of strains #2038, III_B, and III_F did show an EMF-induced increase in population motility over control cells (up to ~20%) at conditions predicted by the "ion cyclotron resonance" model, but this effect was ephemeral. Later, III_B showed a similar increase that was abolished when (1) non-pre-incubated cells were used, (2) the EMF-producing coils were not energized, and (3) even harmonics were used. On observing the response of diatoms to EMFs in real time, a significant increase (~2-fold) in diatom speed over control cells was evident at "ion cyclotron resonance" conditions, using strain #2038 (pre-incubated at 20 μT). The effect was abolished at an even harmonic. We conclude that EMFs can modulate diatom motility, but that the system is, as yet, not consistently reproducible.

Introduction

Despite a vast literature describing the effects of externally applied, non-ionizing, extremely low frequency (< 100 kHz) electromagnetic fields (ELF EMFs) on biological systems, this branch of science is neither well known nor understood. The reasons for this are probably threefold. Firstly, there is no well-accepted mechanism, based on empirical studies, by which EMFs can influence and modify a biological or biochemical process. Any interaction mechanism must describe how fields with energy levels less than that of thermal noise (k_T) can exert an effect (Male, 1992). Secondly, experimentation paying proper attention to both biological *and* physical variables is necessary, but is not common. Thirdly, there is no "tried-and-tested" model system that can be used to test new developments in bioelectromagnetic science. The acceptance of empirical evidence is usually determined by the ability of others to replicate research findings, and investigation to this end has yet to produce a robust system, independently replicable in different laboratories. Here we report attempts to replicate the findings of experiments describing EMF-induced increased motility in populations of the marine diatom *Amphora coffeaeformis* Agardh (McLeod *et al.*, 1987a; Smith *et al.*, 1987a, b).

Amphora coffeaeformis is a common benthic marine pennate diatom (Round *et al.*, 1990) whose motility is well documented (Cooksey and Cooksey, 1980; Round *et al.*, 1990). Motility can be observed by light microscopy in real time and is easily measured on agar plates because the diatoms leave visible trails of mucus. The mucus is produced via a channel, or raphe, running along the long axis of the diatom (Edgar, 1980). In *A. coffeaeformis* the raphe is restricted to one side of the diatom only. Diatoms have many advantages as biological models: they are easy to maintain in culture; have a short generation time (<1 day, Round *et al.*, 1990); are single-celled and eukaryotic; and commonly reproduce asexually, giving a large population of cloned individuals. Motility in *A. coffeaeformis* depends on external [Ca] (Cook-

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sey and Cooksey, 1980), and this property was exploited by McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) who demonstrated that populations of *A. coffeaeformis* on agar containing a [Ca] that normally checked motility could be induced to move when exposed to specific ELF EMFs. Amongst the many studies of bioelectromagnetic effects, these were important because they used a biological system that was potentially easy to re-create in other laboratories, thus allowing replication studies, and they demonstrated that, in common with most bioelectromagnetic effects, a dose-dependent response was not apparent; rather the effect was maximal around specific field amplitudes and frequencies, the latter as predicted by a theoretical model ("ion cyclotron resonance"; Liboff, 1985). In effect, McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) putatively 'tuned' the applied fields to stimulate Ca^{2+} ion activity. Thus the studies provided a clue to the interaction mechanism between living systems and EMFs.

"Ion cyclotron resonance" theory can be used to predict the frequency of EMF that will stimulate any ion, dependent on its charge-to-mass ratio, according to the formula

$$f = \frac{1}{2\pi} \frac{q}{m} B,$$

where f is the stimulation frequency, q/m the charge-to-mass ratio and B the static magnetic field (Liboff, 1985). Extrapolating from the model, stimulation at even harmonics should abolish the effect, and in terms of diatom motility, this was what was found (McLeod *et al.*, 1987b). In addition, McLeod *et al.* (1987b) found that stimulating at "ion cyclotron resonance" conditions for the K^+ ion reduced diatom motility, which again centered on predicted field amplitudes and frequencies.

Clearly the "diatom system" presented a testable model and suggested an interaction mechanism. Not surprisingly, several groups then attempted to replicate the original findings (Reese *et al.*, 1991; Parkinson and Sulik, 1992; Saalman *et al.*, 1992; Prasad *et al.*, 1994; Florig, pers. comm.) and, with one exception, all failed. Reese *et al.* (1991) reported a partial replication in that diatom population motility was increased on EMF stimulation in some, but not all, experiments (15/19 at 0.25 m*M* Ca). Here we report the most thorough attempt at repeating the original findings of the diatom system to date, testing a wide range of both biological and EMF conditions. Our aim is to assess the repeatability of the original experiments, implicating a potential interaction mechanism, and to examine the usefulness of the diatom system as a model for bioelectromagnetic phenomena in general.

Materials and Methods

Because our primary aim was to investigate the repeatability of the results of McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) our methods follow theirs as closely as possible.

Diatoms

Five strains of *Amphora coffeaeformis* were used. Mixed cell-size cultures of strains #2036, #2038, and #2039 were obtained from the Culture Collection of Algae at the University of Texas (Starr and Zeikus, 1987). A mixed cell-size culture of strain III_B (referred to here as III_F) was obtained from the University of Southern California. A narrow size-range (21–23 μm along the long axis) culture of strain III_B was obtained from B. Cooksey at Montana State University. These latter two strains are claimed to be descendants of the strain referred to in the original publications (McLeod *et al.*, 1987a; Smith *et al.*, 1987a, b) on diatom/EMF interaction (B. Cooksey, pers. comm.).

Although these strains are conspecific, there is considerable variation in their gross morphology (Fig. 1); they show differences in upper lethal temperature (Davies, unpubl. data), and they produce differently shaped growth mats in liquid culture (Davies, unpubl. data). Gallagher (1986 and pers. comm.) advocates a reexamination of the taxon *Amphora coffeaeformis* with a view to splitting it into numerous species. Thus, it is important that strain types and their origin be specified in studies such as this (see Wood and Leatham, 1992).

Diatoms were grown in the ASP2 medium of Provasoli *et al.* (1957), modified to contain 0.25 m*M* Ca (Smith *et al.*, 1987a). Reserve stocks of diatoms were kept on 2% w/v Sigma 'A' agar in modified ASP2 slants in polystyrene tubes at 15°C. Diatoms for experimental use were grown either (a) in an incubator at 20°C at 3000 lux (~58.8 μE s^{-1} m^{-2}) illumination from fluorescent lights on a 12-h light-dark cycle where the horizontal component of the magnetic field, B_H = 4.7 μT; the vertical component, B_V = 14 μT; and the oscillating fields were negligible; or (b) in a water bath at 25°C at 3000 lux continuous fluorescent illumination (B_H = 23 μT, B_V = 20 μT; when a thermostatically controlled heater was operating, B_{Hac} = 13 μT peak-peak at 50 Hz, B_{Vac} = 3 μT peak-peak at 50 Hz). Cultures were periodically (about every 4 months) treated with an antibiotic solution (see Stein, 1973) to maintain axenicity.

A second water bath constructed of acrylic plastic with polystyrene insulation was used for EMF pre-incubation experiments and was enclosed by near-Helmholtz coils (see *Exposure apparatus*). Water was pumped to this bath by, and recirculated to, a Lauda-thermostat recirculating water bath sited 1.5 m from the acrylic bath. Diatoms were grown here at 25°C at 1000 lux (~19.6 μE s^{-1} m^{-2}) illumination from DC lamps for at least 1 month prior to experimentation.

Aseptic techniques were practiced throughout.

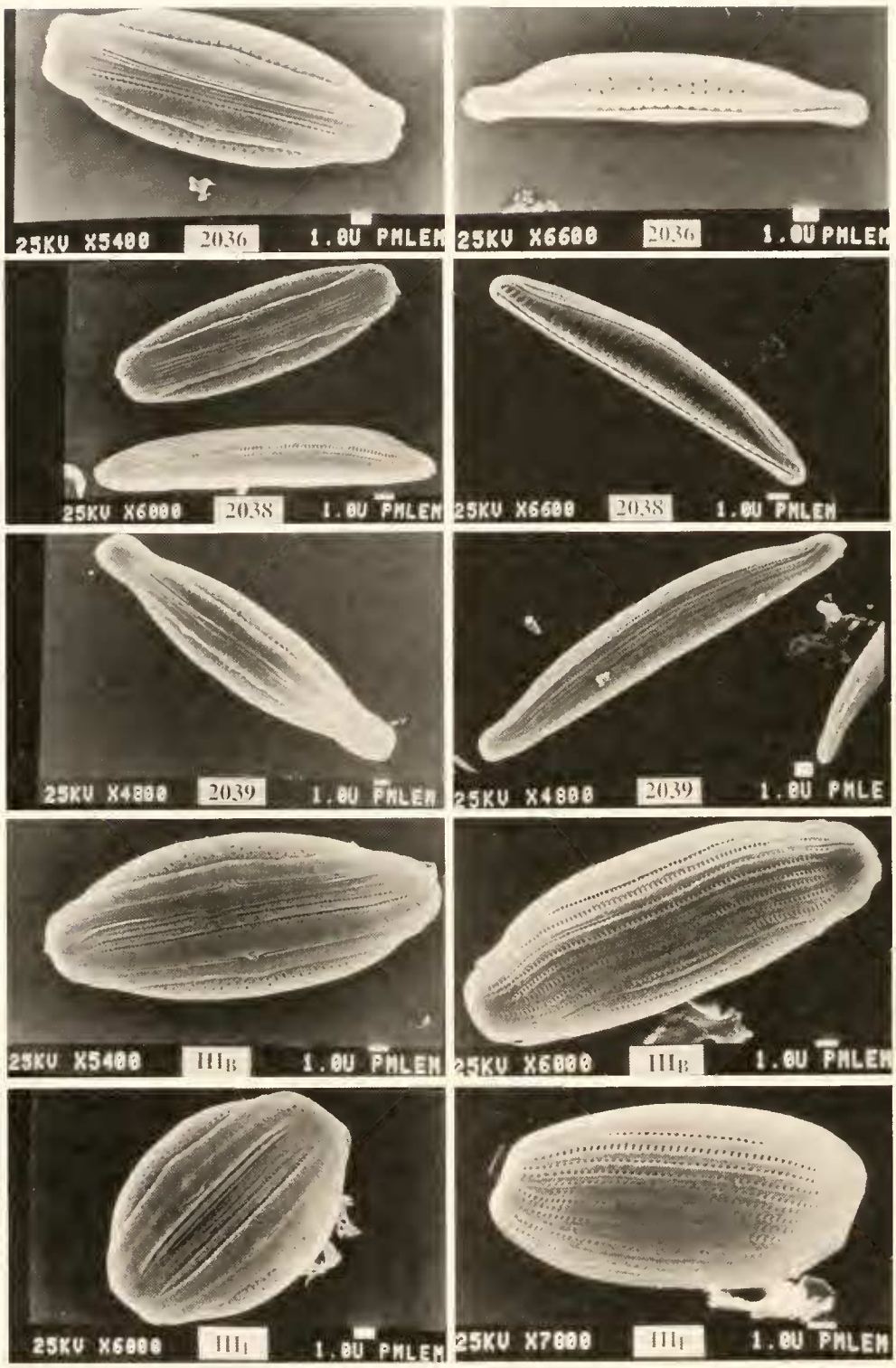

Figure 1. Strains #2036, #2038, #2039, III_B and III_F of *Amphora coffeaeformis*. Left, views of the raphed surface; right, lateral views. Scale bar is 1 μm in each case.

Exposure apparatus

Two pairs of coils (each 295-mm internal diameter) were mounted in a PVC frame at 90° to each other in a near-Helmholtz configuration. One of the pairs (260-mm separation) had its magnetic axis along the z-axis and controlled the vertical (static) component (B_V) of the EMF within the coils. This pair was supplied with current

from a Kingshill 50V2C constant-voltage supply. The second pair (135-mm separation) had its magnetic axis along the *x*-axis (north-south) and controlled the horizontal (both static, B_H, and alternating, B_{Hac}) components of the EMF within the coils. This pair was driven by a Farnell stabilized power supply E30/2 coupled to a Farnell synthesized digital signal generator DSG2 whose outputs were fed through a Hewlett-Packard 6826A bipolar power supply/amplifier acting as a variable gain amplifier. The output of this latter device was used to energize the coil pair. The EMF in the *y*-axis (east-west) was brought to zero by aligning the *x*-axis along the earth's north-south magnetic maximum. Thus we could control the magnitude of the vertical (static) component (B_V), the magnitude of the horizontal static component (B_H), and the magnitude and frequency of the sinusoidally oscillating component (B_{Hac}) of the EMF within the coils.

The EMFs generated were measured at the center of the coils using a Domain SAM3 single-axis fluxgate magnetometer, whose analog display was used to record the static field components. This magnetometer was coupled to an Iso-tech ISR420 oscilloscope that was used to check the magnitude and frequency of the oscillating field components.

Experimental samples were placed at the center of the coils on a platform that could be raised or lowered, depending on the number of samples introduced. The coils were placed on a table at least 1 m away from any metal object. The geomagnetic field components within the coils were identical to those at the site chosen for the control (sham) exposure 2 m away ($B_H = 16.5\ \mu T$, $B_V = 44\ \mu T$). Lighting over both coil and sham sites was provided by DC lamps producing a flux density of 500 lux ($\sim 9.8\ \mu E\ s^{-1}\ m^{-2}$) at sample level. Experimental (room) temperature was 23 ± 1°C. The laboratory chosen for experimental work (at the Plymouth Marine Laboratory, Citadel Hill, Plymouth) was electromagnetically quiet: no EMF interference could be detected at nT levels.

A second set of two coil pairs (each 300-mm internal diameter) was employed in EMF pre-incubation of cells and enclosed a water bath. The coils were mounted in a wooden frame at 90° to each other in a near-Helmholtz configuration. The coils controlling the horizontal field were separated by 150 mm; those controlling the vertical field by 210 mm. The EMF in the *y*-axis was again brought to zero utilizing the geomagnetic maximum. Both coil pairs were driven by a Farnell E30-1BT dual power supply such that the EMF in the center of the coils was set to $B_H = 20\ \mu T$, $B_V = 0\ \mu T$, negligible oscillating components.

Agar plate experiments

There is some confusion over the type of agar used by McLeod *et al.* (1987a) and Smith *et al.* (1987a, b). Although McLeod, Smith, and K. Cooksey (pers. comms.) maintain that Difco Noble agar was used, our experiments with this agar demonstrated that its Ca content was too high to produce the prerequisite Ca-limiting effect on motility (Fig. 2). Figure 2 also shows a Ca-curve prepared using Difco Noble agar from the same container as was claimed to be used in the original experiments (McLeod, Smith, and K. Cooksey, pers. comms.). This latter curve was produced by one of us (MD) on a study visit to Montana State University. Subsequent spectrophotometric analysis showed Difco Noble agar (UK batch) to contain ~62 m*M* Ca. For routine work we have therefore used Sigma 'A' agar (~6 m*M* Ca) which gives Ca-curves consistent with those of McLeod *et al.* (1987a) and Smith *et al.* (1987a, b).

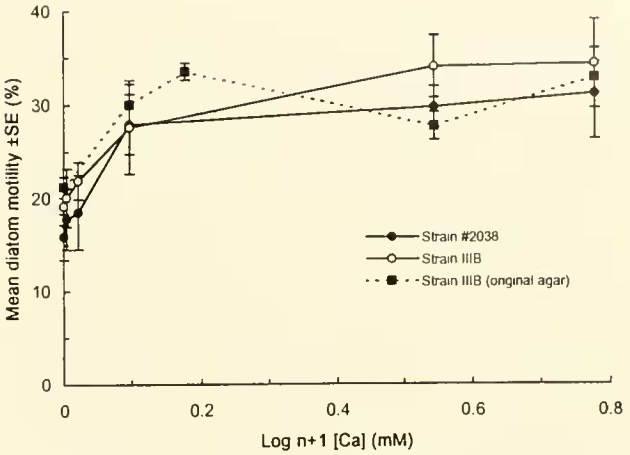

Figure 2. Diatom motility response to external [Ca] on Difco Noble agar. In each case $n = 3$ agar plates with 100 diatoms counted per plate.

Agar (Sigma 'A') was introduced at 2% (w/v) to various maintenance media (the minimal medium [M-M] of Smith *et al.* [1987a]) each containing 308 m*M* NaCl, 8.1 m*M* KCl, 20.2 m*M* $MgSO_4$, 8.3 m*M* Tris-HCl and adjusted to pH 7.6–7.7, but varying in their Ca (as $CaCl_2$) content (0–10 m*M*). After each mixture was autoclaved for 5 min at 121°C, agar plates were made by pipetting 2 ml of the molten agar into each 50-mm-diameter polystyrene petri dish required. Thus a series of plates could be prepared containing 0–10 m*M* added Ca. Plates were wrapped in aluminum foil, stored at 4°C, and used 3–21 days after pouring. Plates used less than 3 days after pouring gave poor diatom tracks.

Diatoms were harvested by centrifugation ($1000 \times g$) for 5 min at incubation temperature before being both washed and recentrifuged twice in Ca-free maintenance medium to ensure a Ca-free pellet of diatoms. The cells were then adjusted, using a hemacytometer, to a density of $1.5 \times 10^5\ ml^{-1}$. In later experiments, in accordance with a revised protocol supplied from Montana State University (McLeod, pers comm.), cells were then returned

to the incubator for 30 min prior to plating. This step was introduced to the revised protocol putatively to allow diatom membranes ruptured during centrifugation to heal (Smith, pers. comm.).

McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) used a paintbrush to introduce diatoms to the agar. The volume of suspension applied in this way was not constant, and it was difficult not to disturb the agar surface. We substituted a technique in which a 5-μl drop of density-adjusted cell suspension was introduced to the agar on each plate at one end of a 30-mm line marked on the underside of the plate. The drop was then drawn along the length of the line with an inoculation loop whose tip had been squared to present a 3-mm bar to the agar surface. Care was taken not to touch the loop to the agar.

After experimental exposure, diatoms on the plates were killed by introducing a few drops of 2% (w/v) OsO_4 to the inside of the lid of each plate. This procedure ensured that there was no additional time after the termination of plate exposure during which the diatoms could move.

Diatom movement on each plate was scored by counting 100 diatoms in random fields viewed with a phase-contrast microscope. Diatoms were scored as motile or nonmotile depending on the presence or absence of a track visible at either pole of the frustule. Only spatially individual diatoms were counted: diatoms that appeared in clumps or whose track originated in a clump were not scored. If these latter diatoms had been scored as motile, then all clumped diatoms would need to be scored as nonmotile. Diatoms were also not scored if they could be clearly seen to be lying with a lateral surface touching the agar. These diatoms are incapable of movement (pers. obs.) because the raphe system is not in contact with the substratum.

(i) Growth curves. Growth curves were produced for each strain of *A. coffeaeformis* by introducing a 1.5×10^5-cell inoculum in modified ASP2 into a polystyrene tube and making the total volume up to 5 ml with modified ASP2. Growth, measured daily by hemacytometer (two chambers counted) was monitored for 8 days in three tubes for each diatom strain. Diatoms were incubated at either 20°C in the incubator or 25°C in the water bath.

(ii) Age-dependent motility. To investigate the relationship between age of culture and diatom motility, cells of each strain were harvested (see above) at 1–8 days after inoculation (1.5×10^5 cells in 5 ml) and streaked onto three agar plates each containing 5 m*M* added Ca. Motility on the plates was scored after 1 h in the incubator at 20°C. Again diatoms were used from both incubator (20°C) and water bath (25°C).

(iii) Ca curves. The relationship between extracellular [Ca] and motility was investigated for all five strains of diatom by preparing a series of agar plates containing a range of [Ca] from 0–5 m*M*. Diatoms of each strain were harvested when in their most motile phase of growth as determined from the results of the age-dependent motility tests (3 days for #2036, #2038, and #2039, and 2 days for III_F and III_B) and introduced to three plates at each [Ca]. Motility was scored after 1 h in the incubator at 20°C. Diatom cultures were grown in the water bath (25°C).

(iv) Effect of light intensity. McLeod (pers. comm.) and Parkinson and Sulik (1992) found that light intensity during diatom exposure influenced the proportion of diatoms showing motility. To test this we harvested a 6-day-old incubator-grown #2038 culture and seeded its diatoms onto 15 agar plates containing 5 m*M* added Ca. Plates, in triplicate, were placed in illumination at <0.625 (inside a drawer), 21.4, 500, 1190, and 2740 lux (<0.01, 0.4, 9.8, 23.3, and 53.7 $\mu E\ s^{-1}\ m^{-2}$, respectively). After 1 h of exposure, the diatoms were killed and scored for motility.

(v) EMF experiments. In all cases we searched for EMF-induced motility using plates with 0.25 m*M* or 0.5 m*M* added Ca. Diatoms in logarithmic growth were seeded onto six replicate plates for each experiment. Three plates served as treatment (EMF-exposed) plates and three as control (sham-exposed) plates. Control and treatment plates for each experiment were always run simultaneously and were seeded from the same diatom culture at its most motile phase of growth (Parkinson and Sulik, 1992). Using diatoms from more than one culture would have introduced confounding factors. A positive control in the form of a plate with 5 m*M* added Ca was always placed with the sham-exposed plates to check that the diatoms were motile at high [Ca]. Each exposure lasted 1 h. To allow for geomagnetic disturbance, experiments conducted on days when large fluctuations in the earth's magnetic field occurred (data from Hartland Monthly Bulletin, British Geological Survey) were repeated at a later date. "Large fluctuations" was arbitrarily defined as a variation greater than 30 nT in either horizontal or vertical intensity during any experimental period.

All strains were exposed to calcium "ion cyclotron resonance" conditions, which produced the greatest field effect for McLeod *et al.* (1987a) and Smith *et al.* (1987a, b). These were: $B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 16 Hz.

Subsequently a set of permutations of field conditions was developed that covered all possible avenues of approach in attempting to reproduce the results of Smith *et al.* (1987a, b) and was designed to allow the detection of any ELF-EMF effect on diatom motility. These conditions were tested both with strain III_F, which was claimed to be the same strain as used by Smith *et al.* (1987a), and strain #2038, with which a partial replication of the original results has been achieved (Reese *et al.*, 1991). Each permutation was tested twice. In all experiments, except

those in (7) below, the vertical component of the earth's field was nulled ($B_V = 0$).

The following permutations were used (the amplitude of B_{Hac} is expressed as peak-peak).

(1) Frequency response. $B_H = 20.9\ \mu T$; $B_{Hac} = 41.8\ \mu T$ at frequencies from 1 to 24 Hz in 1-Hz steps. This was an attempt to demonstrate the frequency window for cell motility shown by Smith *et al.* (1987a).

(2) Amplitude response. $B_H = 20.9\ \mu T$; B_{Hac} varied from 5 to 140 μT (in 5-μT steps to 30 μT, 10-μT steps to 80 μT, and 20-μT steps to 140 μT) at 16 Hz. This was an attempt to demonstrate the amplitude window for cell motility shown by Smith *et al.* (1987a).

(3) Amplitude response. $B_H = (B_{Hac})/2$; B_{Hac} varied from 5 to 140 μT (in 5-μT steps to 30 μT, 10-μT steps to 80 μT, and 20-μT steps to 140 μT) at 16 Hz.

(4) Amplitude response. B_H = ambient; B_{Hac} varied from 5 to 140 μT (in 5-μT steps to 30 μT, 10-μT steps to 80 μT, and 20-μT steps to 140 μT) at 16 Hz.

(5) Amplitude response. $B_H = 0\ \mu T$; B_{Hac} varied from 5 to 140 μT (in 5-μT steps to 30 μT, 10-μT steps to 80 μT, and 20-μT steps to 140 μT) at 16 Hz.

In (2), (3), (4), and (5), plates with 5 m*M* added Ca were also used as treatment and control plates (again in triplicate) in an attempt to reproduce the reduction in motility reported by Smith *et al.* (1987a) at high EMF amplitudes.

(6) $B_H = (B_{Hac})/2$ = ambient at 16, 12.5, and 6.25 Hz. These latter frequencies are the predicted cyclotron resonance frequency and an even harmonic, respectively, of the unhydrated calcium ion in the ambient horizontal field in our laboratory. This follows from Blackman *et al.* (1985) and Leal *et al.* (1986), whose work suggested that the ambient field is important in inducing an EMF effect.

(7) Switched field axes at ion cyclotron resonance conditions. $B_H = 0\ \mu T$; $B_V = 20.9\ \mu T$; $B_{Vac} = 41.8\ \mu T$ at 16 Hz. Switching field axes altered field quantities within the plates (see McLeod *et al.*, 1983).

(vi) Effect of exposure period. McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) used an exposure period of 1 h. In our search for an EMF effect we tried extending this period. We harvested a 6-day-old #2038 culture and a 4-day-old III_F culture (both grown in the incubator) and seeded their diatoms onto six replicate plates with 0.25 m*M* added Ca and one plate with 5 m*M* added Ca. Three of the plates with 0.25 m*M* added Ca plus the plate with 5 m*M* added Ca were placed at the sham-exposure site; the remainder were placed at the EMF-exposure site and subjected to Ca ion cyclotron resonance EMF conditions. At 1, 2, 4, 6, 8, 12, 16, 24, 34, and 58 h after the exposure commenced, each plate was removed from exposure or sham-exposure, its diatoms were scored for motility as above, and the plate was returned to the exposure or sham-exposure site.

(vii) Effect of cell density. To test whether the density of the diatom cells on the agar plates had an effect on motility or susceptibility to EMFs (see Aarholt *et al.*, 1981; Carson *et al.*, 1990), a 6-day-old incubator-grown #2038 culture was harvested and its cells were seeded onto 5 plates with m*M* added Ca. Quadruplicate plates were prepared at each cell density used (5×10^4, 1×10^5, 1.5×10^5, 1×10^6, 2×10^6 cells ml^{-1}). Two of each four were placed in Ca ion cyclotron resonance EMF conditions and two were sham-exposed. After 1 h of exposure, the diatoms were killed and scored for motility.

(viii) Effect of position in cell cycle. To test for EMF susceptibility of diatoms at different stages of their cell cycle, cells (III_F) were acclimatized (2 weeks) to a regime of 12 h light/dark at 20°C in the incubator. Following this, on one day cells were inoculated in the usual manner into polystyrene tubes at intervals of 2 h for a total of 16 h. Each culture was then simultaneously harvested 4 days later when in logarithmic growth and seeded on to triplicate sham-exposed and triplicate Ca ion cyclotron resonance-exposed plates with 0.5 m*M* added Ca plus a plate with 5 m*M* added Ca as a positive control. After 1 h of exposure, the cells were scored in the usual way.

(ix) Effect of EMF on distances moved. During the frequency-response test above, the distances moved by III_F cells during the 1-h exposure period were recorded using a calibrated eyepiece graticule attached to the microscope. The distances moved by 10 motile diatoms were recorded on each triplicate sham-exposed and each triplicate EMF-exposed plate (each 0.5 m*M* added Ca) in each experiment. The frequency of B_{Hac} was varied from 1 to 24 Hz in 1-Hz steps, $B_{Hac} = 41.8\ \mu T$, $B_H = 20.9\ \mu T$, $B_V = 0$.

(x) Effect of EMF on direction of movement. The direction of cell movement was also recorded during the frequency-response test using strain III_F. An eyepiece graticule was divided into twelve 30° sectors. Each motile diatom scored was assigned to a sector depending on its initial direction of movement (0° = north). In this way, 100 diatoms per triplicate plate with 0.5 m*M* added Ca were scored in EMF-exposure and sham-exposure conditions at each frequency tested. Direction of movement was tested for nonrandomness using χ^2 tests.

(xi) Effect of EMF pre-incubation. After at least a month of pre-incubation (subculturing every 4–5 days) in a water bath at $B_H = 20\ \mu T$ (see *Exposure apparatus*), 1-, 2-, 3-, 4- and 5-day-old cells of each strain of *A. coffeaeformis* were exposed at Ca ion cyclotron resonance EMF conditions. Exposures lasted for 1 h on triplicate agar plates for each treatment. Treatment plates ranged from 0 to 5 m*M* in added Ca. Those experiments that indicated a positive response to the EMFs in terms of motility were repeated. Five replicate experiments were

Figure 3. Equipment used in perfusion experiments. Near-Helmholtz coils arranged around microscope stage. Video display of diatoms in perfusion chamber.

also conducted with 3-day-old diatoms (in logarithmic growth) of strain III_B under three conditions: (1) using cells that were not pre-incubated (grown at ambient: B_H = 16.5 μT, B_V = 44 μT); (2) where the near-Helmholtz coils were disconnected from their supply (which remained switched on); and (3) at the even harmonics of 8 and 32 Hz (other field conditions remained the same). According to Smith *et al.* (1987a), shifting to even harmonics should abolish any EMF effect. The experiments were conducted not in the order listed but in a random order, determined using random number tables.

Perfusion experiments

All perfusion experiments were performed on cultures of strain #2038 that had been incubated at B_H = 20 μT for at least one month (see above). Perfusion media were at room temperature (23 ± 1°C).

(i) Equipment. The near-Helmholtz coils used for experimental EMF exposure were placed around a Reichert Zetopan microscope so that the microscope stage was at the center of the coils. The microscope was set for phase contrast and was linked to a U-matic video recorder and monitor *via* a Panasonic CL700 CCD video camera (Fig. 3). The coils were again aligned along the geomagnetic maximum (see above) and, when energized, the field at stage level was at Ca ion cyclotron resonance conditions. The sensing head of the fluxgate magnetometer (56 × 48 × 22 mm) was too large to fit on the stage with the objective lens cluster in place, so the latter was removed during EMF measurement. However, the introduction of the objective lens cluster next to the sensing head within energized coils caused no more than 1 μT deviation in magnetic flux density. During perfusion, diatoms were viewed through a long-working-distance 20× phase-contrast objective lens and displayed on the video monitor.

A perfusion chamber was created from two small glass plates separated by a 30-mm diameter O-ring and clamped together with bulldog clips. Two hollow (hypodermic) needles (0.55-mm diameter) were inserted through the O-ring at points 180° to each other to act as fluid entry and exit ports. Polyethylene tubing was connected to these ports. Two 60-ml syringes acted as reservoirs from which perfusion fluid flowed under gravity through silicone rubber tubing and then polyethylene tubing to the perfusion chamber on the microscope stage. Flow from each reservoir was controlled using a clip on the silicone rubber tubing. An acrylic plastic junction unit allowed flow into the chamber from one reservoir only. In a chamber flooded with water, the time taken from switching to a

flow of food coloring until the central area of the chamber was flooded with color was 50 s.

(ii) Effects of Ca levels on motility. Since the examination of diatom motility in a perfusion chamber was a novel technique, we initially assessed the effects of [Ca] in the perfusion medium. Three-day-old cells were harvested by a single centrifugation (5 min at 1000 × *g*) at 25°C and resuspended in 5 m*M* added Ca minimal medium (M-M). A drop of this cell suspension was introduced into the center of the perfusion chamber. Following a 5-min period allowing for diatoms to adhere to the glass substratum, the chamber was perfused for 10 min with 5 m*M* added Ca M-M. Perfusion was then switched to 0 m*M* added Ca M-M for 5 min; switched back to 5 m*M* added Ca M-M for 5 min; to 0 m*M* added Ca M-M for 5 min; and finally to 5 m*M* added Ca M-M for 5 min. Video recordings of one field of view were made during the whole of this procedure. Diatom speed was estimated by measuring, on screen, the distance moved by each motile diatom in the 10-s period prior to each switching of perfusion medium and in each 10-s period following 1 min of perfusion (except the initial perfusion with 5 m*M* added Ca M-M).

(iii) EMF experiments. The procedure for EMF experiments closely followed the Ca-response experiments above. Cells (3 days old) were again pelleted by a single centrifugation and resuspended in 5 m*M* added Ca M-M. A drop of diatom suspension was introduced into the perfusion chamber. Perfusion with 5 m*M* added Ca M-M commenced after 10 min. Diatoms that showed motility were then observed during the remainder of the procedure. After 10 min, perfusion was switched to 1 m*M* added Ca M-M (a Ca level suboptimal for diatom motility). Motility was measured as before, after 2 min. The EMFs were applied and motility was remeasured after a further 3 min. EMFs were then discontinued and motility was remeasured after a further 3 min.

The above procedure was repeated until a total of 30 diatoms had been recorded during each treatment, with 3–10 diatoms (depending on the number visible on the monitor screen) recorded during each procedure. Control experiments were performed by following the procedure but not applying any EMF. Each treatment and its control were performed during one 3-h period on diatoms from the same culture. Two treatments were used. One employed EMFs at Ca ion cyclotron resonance conditions. The second (conducted 2 days later) involved a manipulation of frequency to an even harmonic which should, according to ion cyclotron resonance theory, abolish any EMF effect. We used $B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 32 Hz—*i.e.*, the same conditions for ion cyclotron resonance but at double the frequency.

Paramagnetic investigations

To assess whether the response of diatoms to EMFs was owing to paramagnetism, each strain of *A. coffeaeformis* was grown in polystyrene tubes or glass flasks from 30 000 cells ml^{-1} until stationary phase was attained. Diatoms were harvested by centrifugation (1000 × *g* for 5 min) a minimum of 2 weeks after inoculation and were then washed and recentrifuged four times in deionized (17.4 $M\Omega\ cm^{-1}$) water. Diatom pellets were then lyophilized for 2 days. At no time did the diatoms come into contact with any metal object. To minimize contamination from ferrous particles, both the modified ASP2 and the deionized water used were kept in glass flasks atop a large strong permanent magnet for at least 1 month prior to use (a technique employed by J. Kirschvink, pers. comm.). These liquids were carefully pipetted from the flasks for use.

Samples of freeze-dried diatoms of all strains were sent for analysis to the laboratory of Dr J. Kirschvink (University of California Institute of Technology, USA) where a superconducting quantum interference device (SQUID) was used to detect the presence of biogenic magnetite or ferrous material in the diatoms. For further information on this method, see Kirschvink *et al.* (1992). We had hoped to obtain a sample of diatoms from the group (Reese *et al.*, 1991) that achieved a partial replication of the results of McLeod *et al.* (1987a) and Smith *et al.* (1987a, b), but none were available.

Results

Agar plate experiments

(i) Growth and motility. During growth in culture, each strain of diatom exhibited maximal motility on agar during the logarithmic phase of its growth. This was true whether cultures were grown at 20°C (Fig. 4) or 25°C (Fig. 5), although the time taken to reach logarithmic growth, and hence maximal motility, was shorter at 25°C than at 20°C. These data were used to determine when to harvest cells for maximal motility in the rest of the experiments.

The maximum motilities of #2036, #2038, and #2039 seem independent of growth temperature, whereas those of III_B and III_F increase with increased temperature (maximum mean motilities: III_B at 20°C = 51% ± 7 SE, 25°C = 78% ± 9 SE; III_F at 20°C = 42% ± 4 SE, 25°C = 72% ± 6 SE). The maximum motility recorded on any agar plate during the experiments was for III_B (98%). The higher growth temperature also apparently allows a greater carrying capacity for each strain in culture. Within each temperature treatment the growth curves can be split into two groups: III_B and III_F showed a short logarithmic phase and a small carrying capacity; #2036, #2038, and #2039 showed a longer logarithmic phase and a higher carrying capacity (Figs. 4 and 5).

(ii) Ca curves. In Figure 6, the motility response of our diatoms to external [Ca] is compared with the results obtained by Smith *et al.* (1987a). Our results show that

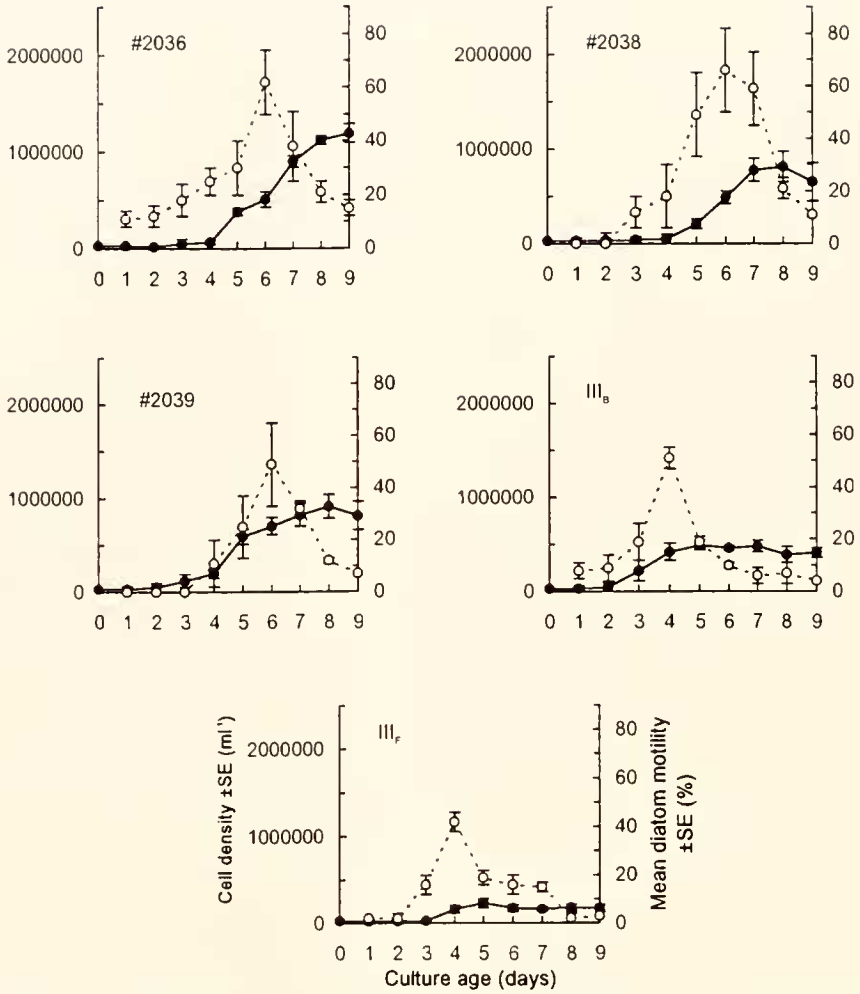

Figure 4. Growth (unfilled circles) and motility (filled circles) of five strains of *Amphora coffeaeformis* at 20°C. In each case $n = 3$.

A. coffeaeformis is incapable of movement in a medium without calcium and that motility increases sharply between 0.25 and 2.5 m*M* external [Ca]. The motility curves for strains III_B and III_F were very similar and showed saturation (of ~70% motility) at about 5 m*M* external [Ca]. The other strains did not show saturation. Strains #2036 and #2038 showed a similar motility response, rising to ~60% at 5 m*M* external [Ca], and #2039 gave a relatively poor response to millimolar Ca levels (40.1% at 5 m*M* external [Ca]). In each case the motility on plates with 0.25 m*M* added Ca was of the order of a few percentage points. This is a prerequisite for the detection of an EMF effect with this system.

(iii) Effect of light intensity. Varying the photon flux density from <0.01 to 53.7 $\mu E\ s^{-1}\ m^{-2}$ had no effect on the motility of #2038 diatoms (Table I).

(iv) EMF experiments. The results of EMF experiments are presented in Tables II–XII. Most data were analyzed by *t*-tests as pairs of control and EMF-exposed groups. Most data were not suitable for multivariate or factorial analyses as cell motility in each experiment was largely determined by the position of the diatoms in their growth curve. Although a very broad range of EMF conditions were employed—including those purported to produce ion cyclotron resonance—and in most cases each was tested twice, a significant difference in diatom motility between exposed and control plates was not demonstrated in any case. No amplitude or frequency window was found. There was no reduction in motility at high EMF amplitudes (Tables IV–VII).

For diatoms of strains #2038 and III_F, extending the exposure period up to 58 h did not produce an EMF effect at ion cyclotron resonance conditions (Fig. 7). Diatom motility reached a maximum within 12 h of exposure.

Varying the cell density of #2038 from 5×10^4 to $2 \times 10^6\ ml^{-1}$ prior to plating had no effect on the motility of either control diatoms or those exposed to ion cyclotron resonance EMFs (Table IX). Similarly, the position of

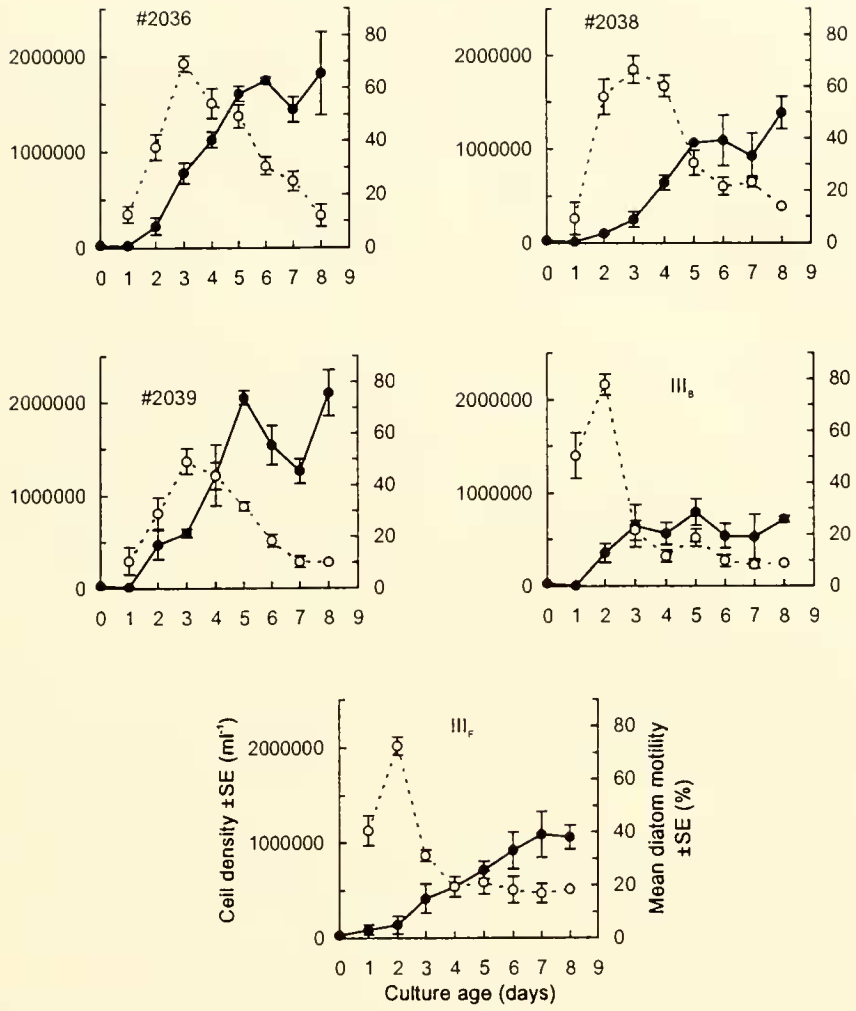

Figure 5. Growth (unfilled circles) and motility (filled circles) of five strains of *Amphora coffeaeformis* at 25°C. In each case $n = 3$.

III_F diatoms in their cell cycle had no effect on population motility at ion cyclotron resonance EMFs (Table X).

The overall mean distance moved by motile III_F diatoms in 1 h was 36.1 μm ± 0.8 SE, $n = 1440$. Neither the presence of an EMF nor its frequency had an effect on distance moved (Table XI). Table XII shows that EMFs at frequencies from 1 to 24 Hz could not elicit directionality in the movement of III_F diatoms. In each case, the initial direction of diatom movement was randomly distributed about the compass.

(v) Effect of EMF pre-incubation. When diatoms (all strains) were tested under Ca ion cyclotron resonance conditions, the initial experiments showed a poor motility response to Ca; this proved to be a result of technical difficulties with cell culture. However, if the EMF had no effect, we would expect that the number of EMF-exposed/control pairs of cell counts showing an increase in cell motility would, on average, equal the number showing a decrease. A significant proportion (71%) of count pairs showed an increase in cell motility on EMF-exposure ($\chi^2 = 57.4$, $P < 0.001$, $n = 322$), although this increase was usually <10% in population motility.

When a good motility response had been established, three strains aged 2 days or older (cultures tested at ages of 2–5 days) showed a positive response to the Ca ion cyclotron resonance EMFs when Ca-dependent motility curves were constructed (Fig. 8). The most marked responses (~20% increase in cell motility) were shown by 2- and 3-day-old cultures of strain #2038 on agar with 2.5 m*M* added Ca. Smaller increases were also shown by older #2038 cultures and by strains III_B and III_F. Strains #2036 and #2039 did not show a positive EMF-response. Our culture of #2038 then went into decline, and fresh material from a reserve stock was pre-incubated at $B_H = 20\ \mu T$, $B_V = 0\ \mu T$ for 1 month. In a further three sets of experiments, this fresh #2038 responded positively to EMF (Fig. 9); however, subsequent attempts, including experiments with the other strains, showed no effect. The greatest positive effect

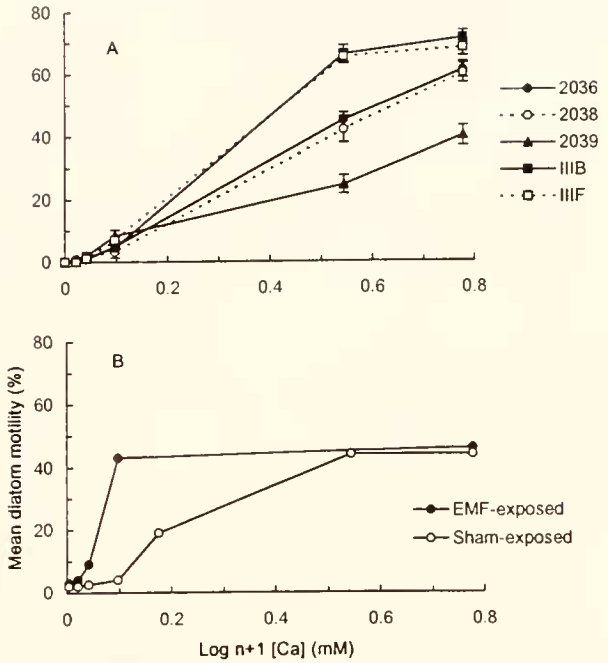

Figure 6. Motility response of *Amphora coffeaeformis* in logarithmic growth to external [Ca]. (A) Present results using five strains of *A. coffeaeformis* (bars are SE, $n = 3$). (B) Results of Smith *et al.* (1987), including an EMF-induced effect for comparison.

(~30% increase in motility) was shown by a 1-day-old culture on plates with 2.5 m*M* added Ca (Fig. 9).

Material from reserve stocks was again pre-incubated at $B_H = 20\ \mu T$, $B_V = 0\ \mu T$, this time for at least 3 months. Strain #2038 again declined, but III_B grew as expected and was used in the following experiments (each repeated five times). Under ion cyclotron resonance conditions, each experiment showed an EMF effect (Fig. 10), the maximum difference in motility between exposed and sham-exposed cells occurring at 2.5 m*M* added Ca (mean increase = +22.1% ± 1.8 SE, $n = 15$). No experiment at Ca ion cyclotron resonance conditions failed to show an EMF effect (Fig. 10), although the percentage shift in motility was smaller, and the [Ca] at which an effect occurred was higher, than in the other experiments using pre-incubation reported above. The EMF effect was abolished when diatoms were grown in the earth's field prior to experimentation (Fig. 11: mean increase in motility at 2.5 m*M* added Ca = −1.8% ± 1.8 SE, $n = 15$) and when the coils were not energized during experimentation (Fig. 12; mean increase = −3.4% ± 0.9 SE, $n = 15$). The effect was also abolished when even harmonics at 8 Hz (mean increase = +1.4% ± 1.1 SE, $n = 15$) and 32 Hz (mean increase = −0.8% ± 1.5 SE, $n = 15$) were used (Figs. 13 and 14). Experimentation was recommenced after a break of 1 month and subsequent experiments, under conditions above that produced an effect, indicated no EMF effect.

Perfusion experiments

(i) Effects of Ca levels on motility. In conditions of 5 m*M* added Ca M-M, all observed diatoms were motile. When the perfusion medium was switched to 0 m*M* added Ca M-M, all diatoms either stopped moving or greatly slowed their movement. When switched back to 5 m*M* added Ca M-M, the diatoms resumed motility. A further switching to 0 m*M* added Ca M-M abolished or reduced motility in all diatoms. Example observations are shown in Figure 15. All diatoms observed regained motility in 5 m*M* added Ca M-M at the end of the procedure.

(ii) EMF experiments. At ion cyclotron resonance conditions (at 16 Hz), a significant increase (approximately 2-fold, over the control) in diatom speed was ob-

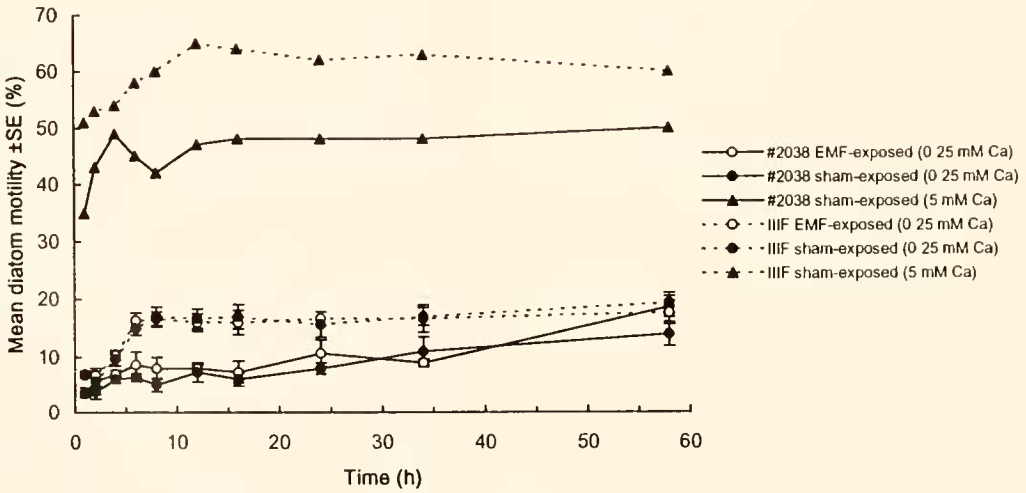

Figure 7. Effect of exposure period on susceptibility of *Amphora coffeaeformis* strains #2038 and III_F to EMFs at ion cyclotron resonance conditions on agar plates. [Ca] refer to levels of added Ca in agar; $n = 3$, except for 5 m*M* Ca plates where $n = 1$.

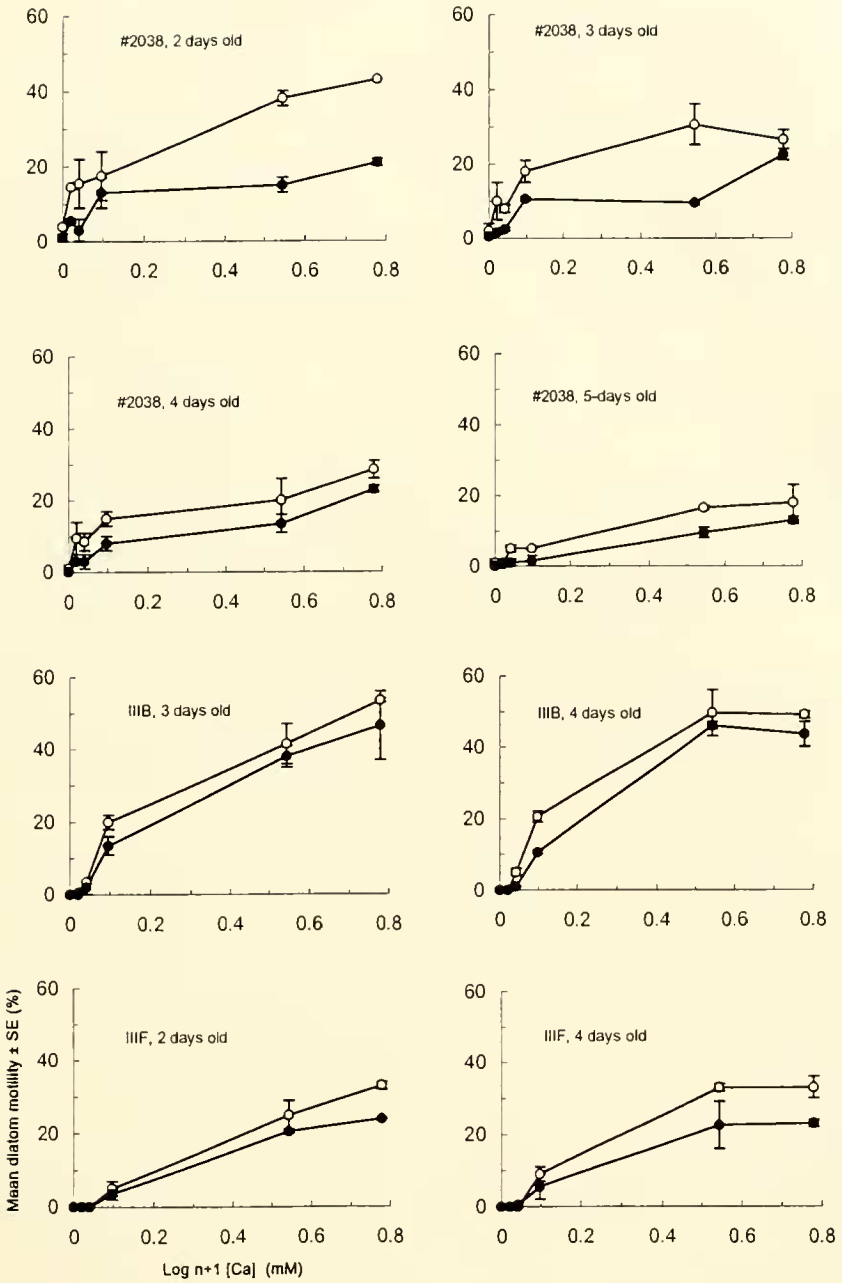

Figure 8. Motility response of strains #2038, III_B, and III_F (pre-incubated at $B_V = 0$, $B_H = 20\ \mu T$ for at least 1 month) to external [Ca] in EMF experiments at ion cyclotron resonance conditions ($B_V = 0$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 16 Hz). Unfilled circles = EMF-exposed; filled circles = sham-exposed; $n = 3$ in each case. Time indicated is culture age. Results shown only where an EMF-induced effect is apparent. Strains #2036, #2039, III_B at 2 and 5 days old, and III_F at 3 and 5 days old showed no effect.

served on application of the EMFs (Mann-Whitney test, $U = 286$, $P = 0.012$; Fig. 16). Such an increase was not apparent either before or after application of EMFs in comparison with the controls (Mann-Whitney tests: $U = 416$, $P = 0.548$; $U = 358$, $P = 0.153$, respectively, Fig. 16); and the pre-exposure, during-exposure, and post-exposure speeds of the control diatoms were not significantly different from each other (Kruskall-Wallis test, $H = 1.44$, $P = 0.486$). A shift to an even harmonic (32 Hz) abolished the EMF effect (Fig. 16): there was no significant difference in speed between diatoms exposed to the EMF and control diatoms either before ($U = 411$, $P = 0.513$), during ($U = 432$, $P = 0.758$), or after ($U = 424$, $P = 0.664$) EMF application. Again the control diatoms showed no significant variation in speed during the experiment ($H = 1.5$, $P = 0.472$).

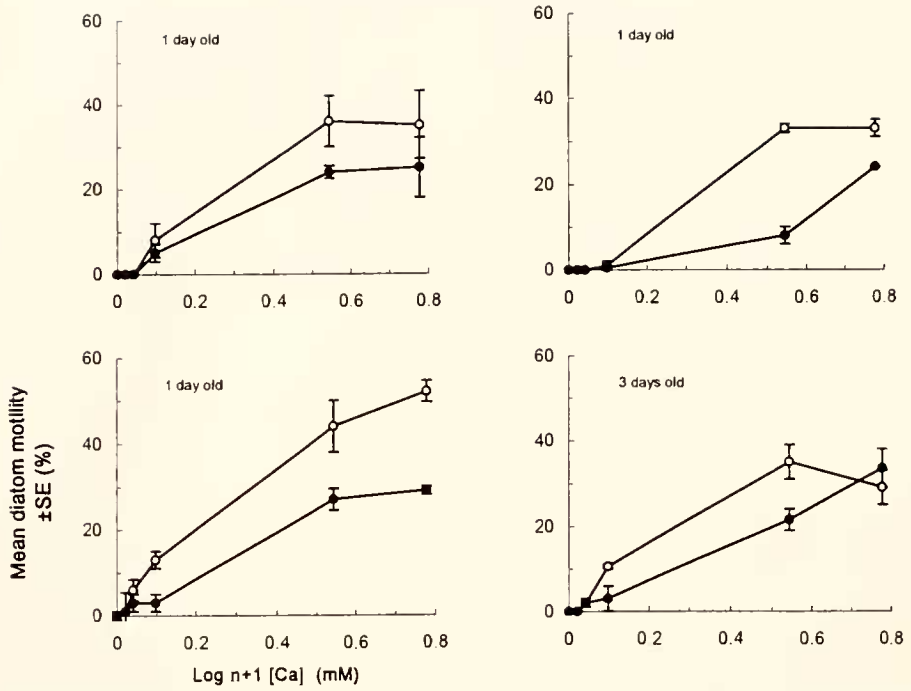

Figure 9. Motility response of strain #2038 (pre-incubated at $B_V = 0$, $B_H = 20\ \mu T$ for at least 3 months) to external [Ca] in EMF experiments at ion cyclotron resonance conditions ($B_V = 0$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 16 Hz). Unfilled circles = EMF-exposed; filled circles = sham-exposed; $n = 3$ in each case.

Paramagnetic investigations

The diatoms exhibited no detectable ferromagnetic properties.

Discussion

In the exploration of the interaction between biological systems and EMFs, the use of diatoms and the 'diatom system' was originally seen as potentially reproducible in different laboratories. The system was heralded as one that could effectively and easily demonstrate an EMF effect (McLeod *et al.*, 1987a, Smith *et al.*, 1987a, b). In addition, by indicating effects only at specific frequency-windows and amplitude-windows, the results of McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) pointed to an interaction mechanism (originally ascribed to ion cyclotron resonance), and this is probably the reason that so many (Reese *et al.*, 1991; Parkinson and Sulik, 1992; Saalman *et al.*, 1992; Prasad *et al.*, 1994; Florig, pers. comm.) have attempted to repeat the original work. However, as we and many of those who have attempted replication have found, the system is more complex than originally thought.

It is apparent from Figures 1, 4, and 5 that the different strains of *A. coffeaeformis* vary considerably in morphology and physiology (see also Parkinson and Sulik, 1992). There are also variations in upper lethal temperature and in the shape of the growth mat in liquid culture (Davies, unpubl. data). Clearly the classification of the diatoms we used as strains or variants of *A. coffeaeformis* is in doubt and there is a need to reexamine the taxon *Amphora coffeaeformis* (Gallagher, 1986, 1987). The variations may in part be owing to the culturing of diatoms over many years in laboratories where selection pressures may differ, and to the process of subculturing in which a small number of cells (often only one) is used to start a new population, producing genetic bottlenecks. In only one (Saalman *et al.*, 1992) of the other attempts to reproduce the findings of McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) were the diatoms of the same strain as used in the original experiments, and this may contribute to a lack of repeatability. If the diatom system is indeed dependent on the use of a single strain of one species, or possibly, the presence of a single gene (which might be lost during subculturing), then its value as a reproducible system is in doubt. Large genetic variation in intraspecific variants of photosynthetic microbes is an acknowledged problem for experimentalists (see, for example, Gallagher, 1980; Medlin *et al.*, 1996; Paasche *et al.*, 1996).

Diatom motility shows a sharp peak during exponential growth (Figs. 4 and 5), and although the physiological explanation for this remains unknown, diatoms clearly must be in exponential growth (show motility) before they can display sensitivity to EMFs. Whether this was the case in the other attempts at replication is not apparent (Reese *et al.*, 1991; Parkinson and Sulik, 1992; Saalman *et al.*, 1992; Prasad *et al.*, 1994). McLeod *et al.* (1987a)

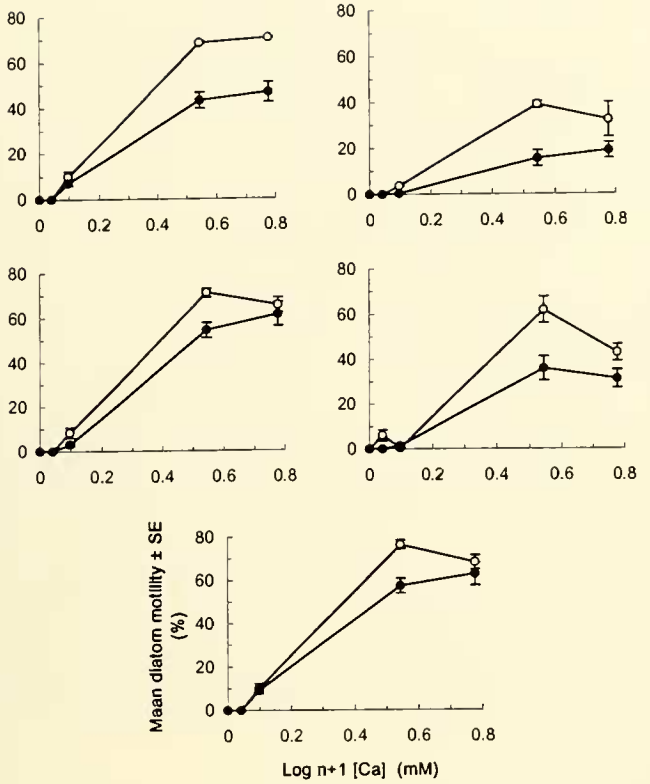

Figure 10. Motility response of strain III_B (pre-incubated at B_V = 0, B_H = 20 μT for at least 3 months) to external [Ca] in EMF experiments at ion cyclotron resonance conditions (B_V = 0, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 16 Hz). Unfilled circles = EMF-exposed; filled circles = sham-exposed; n = 3 in each case.

and Smith *et al.* (1987a) observed a maximum motility of ~45% within any diatom population, ascribing this to the presence of the raphe system on one diatom valve only. We (and Reese *et al.*, 1991, and Saalman *et al.*, 1992) noted that population motility can exceed 50%, perhaps as a result of our plating technique, which allows time for the diatoms to orient themselves in liquid culture before they contact the surface of the agar. In our motility assays, in common with Parkinson and Sulik (1992) and Saalman *et al.* (1992), we counted only diatoms that were not in contact with any others. This condition was set because motility in one diatom may trigger motility in an adjoining diatom, thus producing non-independence of measurements (Saalman *et al.*, 1992). McLeod *et al.* (1987a) and Smith *et al.* (1987a, b) counted some diatoms that were in clumps and some counts may have included (immotile) diatoms whose lateral surfaces were in contact with the agar (Smith, pers. comm.). Thus, it is not surprising that our Ca curves (Fig. 6) do not agree with those of McLeod *et al.* (1987a) and Smith *et al.* (1987a, b). Nevertheless, although diatoms of strains III_B and III_F showed greater motility than those in the original experiments, the shape of the Ca response curves is similar, supporting the contention that these strains are descendants of diatoms used in the original experiments. Cooksey and Cooksey (1980), Parkinson and Sulik (1992), and McLeod (pers. comm.) observed a reduction or abolition of diatom population motility in the dark. We (Table I), on the other hand, in common with Saalman *et al.* (1992), observed motility in the dark, suggesting the existence of a store of energy in diatoms (probably as a pool of ATP). Again our results may be different because we may have used a different strain of *A. coffeaeformis* (#2038) from those of Cooksey and Cooksey (1980) (presumably III_B) and Parkinson and Sulik (1992) (strain not indicated).

Experiments using externally applied EMFs (Tables II–X) showed wide variability in the proportion of diatoms exhibiting motility under putatively the same control conditions. This we can only ascribe to slight variations in population age and culture conditions, of diatoms, which could produce diatoms at slightly different stages of the growth-cycle at the start of the experiments. Clearly control experiments must be concurrent with exposure experiments in this system. Our initial inability to demonstrate an EMF effect using a wider range of field conditions than any previously published, extending the period of exposure, varying the

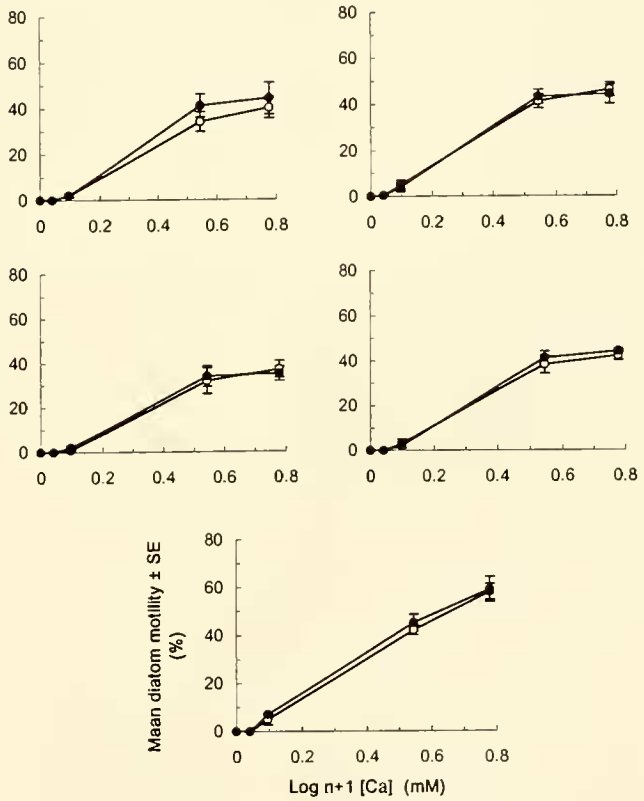

Figure 11. Motility response of strain III_B to external [Ca] in EMF experiments at ion cyclotron resonance conditions (B_V = 0, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 16 Hz). Unfilled circles = EMF-exposed; filled circles = sham-exposed; n = 3 in each case. Diatoms were not pre-incubated before use, but were grown in ambient EMF.

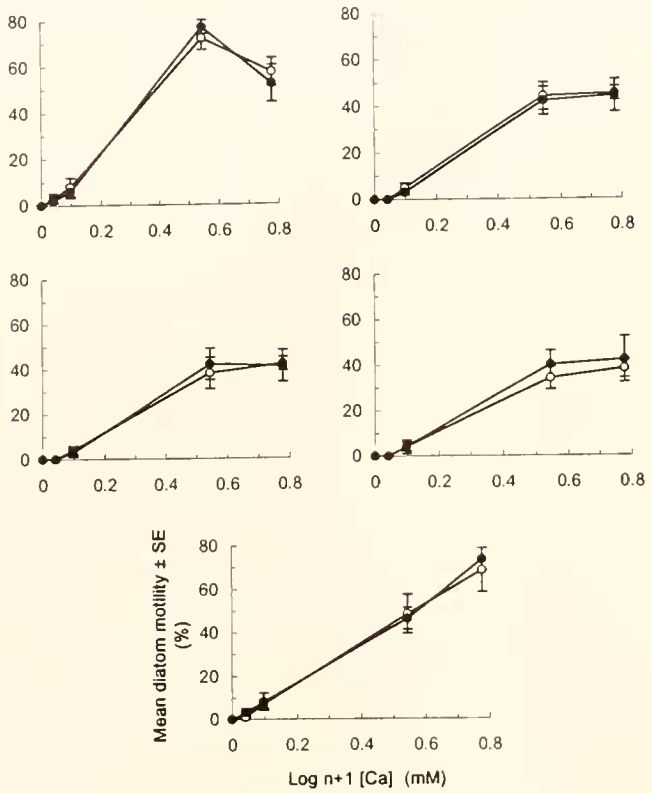

Figure 12. Motility response of strain III_B (pre-incubated at B_V = 0, B_H = 20 μT for at least 3 months) to external [Ca] in EMF experiments in which electrical equipment was set to give ion cyclotron resonance conditions (B_V = 0, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 16 Hz), but the coils were not energized. Unfilled circles = EMF-exposed; filled circles = sham-exposed; n = 3 in each case.

cell density and position in the cell-cycle, and assessing the distance and direction moved by diatoms militates against the potential of diatoms as a useful model for EMF effects. However, the increase (compared to paired controls) in population motility exhibited by a significant proportion of EMF-exposed diatoms (all strains) on agar at ion cyclotron resonance conditions indicates that diatoms are susceptible to the effects of EMFs, but only when they have experienced a defined EMF environment prior to experimentation. Significant increases in motility of EMF-exposed over sham-exposed diatoms were observed in single experiments (Figs. 8–10), although again only where diatoms had been "pre-incubated" in specific EMFs. The abolition of the effect when the EMF-producing coils were disconnected (Fig. 12) gives clear evidence for the role of extraneous fields in producing the effect, and the abolition at even harmonics (Figs. 13 and 14) does not rule out the presence of a frequency window. Blackman *et al.* (1985, 1990) and Leal *et al.* (1986) reported EMF effects that were modulated by the intensity of the geomagnetic field. In our experiments we effectively modified the Earth's field to create defined conditions ($B_H \cong B_H$ used in the experiments at ion cyclotron resonance conditions) during diatom growth, as originally suggested by Smith *et al.* (1987b). Neither we nor Smith *et al.* (1987b) offer an explanation for this phenomenon, but it is unlikely to be owing to any paramagnetism within the diatoms because none was detected. Clearly our initial failure (and that of others) to replicate the original findings may be owing to a lack of appreciation of the effect of the Earth's field. Before using the pre-exposure system, we grew diatoms in conventional incubators with complex spatiotemporal EMF conditions, and a lack of reproducibility of EMF effects in general may be caused by the complex EMFs to which cells and organisms are exposed *prior* to experimentation. Our results support those of Blackman *et al.* (1988) in suggesting that prior treatment of experimental organisms is an important variable in bioelectromagnetic science. Even our experiments involving ambient fields (Table VIII) used diatoms grown in a conventional incubator. The ability of McLeod *et al.* (1987a), Smith *et al.* (1987a, b), and many other workers to observe a response to EMFs without pre-incubation of the test organism may be owing to the continued culture, over many generations, of the test organism in the environment in which experiments are subsequently performed; again, however, the rationale for this effect is not apparent.

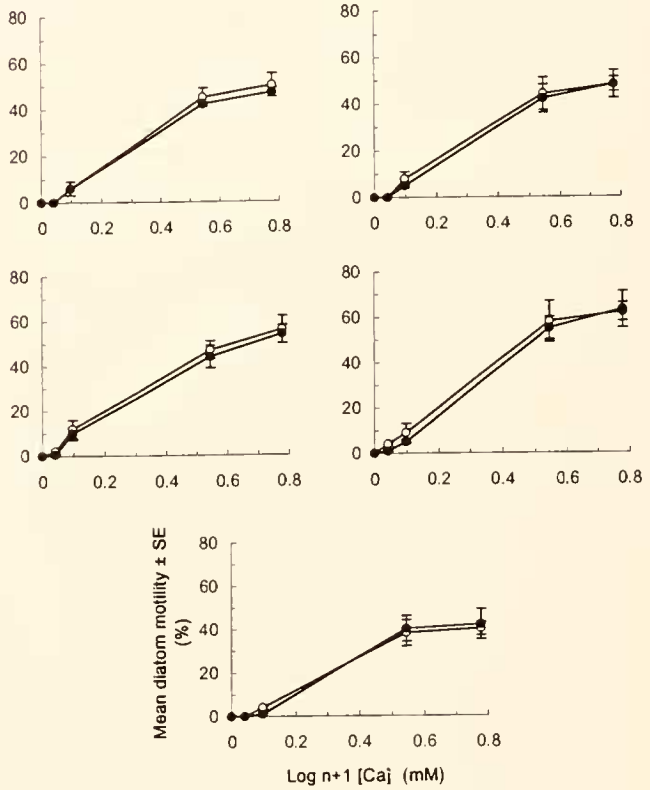

Figure 13. Motility response of strain III_B (pre-incubated at B_V = 0, B_H = 20 μT for at least 3 months) to external [Ca] in EMF experiments at an even harmonic of ion cyclotron resonance conditions (B_V = 0, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 8 Hz). Unfilled circles = EMF-exposed; filled circles = sham-exposed; n = 3 in each case.

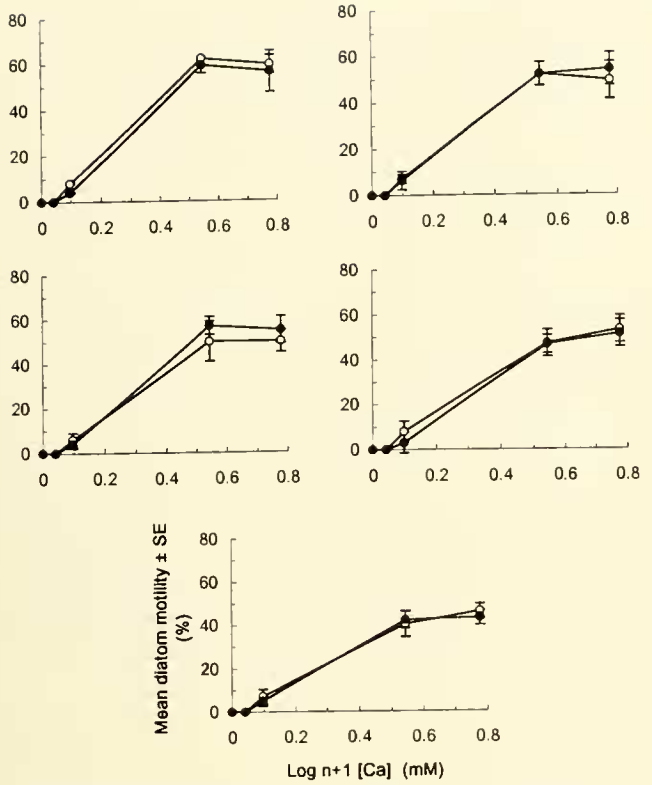

Figure 14. Motility response of strain III_B (pre-incubated at B_V = 0, B_H = 20 μT for at least 3 months) to external [Ca] in EMF experiments at an even harmonic of ion cyclotron resonance conditions (B_V = 0, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 32 Hz). Unfilled circles = EMF-exposed; filled circles = sham-exposed; n = 3 in each case.

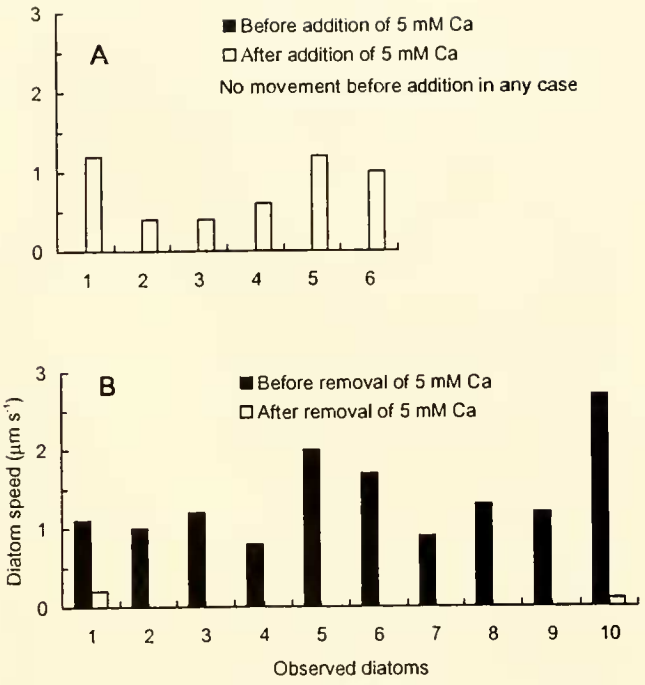

Figure 15. Mean (±SE) diatom speed recorded in real time on addition (A) and removal (B) of 5 mM Ca to the perfusion chamber. Measurements made in 10-s period prior to addition or removal and in 10-s period 1 min after addition or removal (see text).

When the pre-incubation step was used, three strains of *A. coffeaeformis* (#2038, III_B, and III_F) showed a population motility response to EMFs. Strains III_B and III_F might be expected to respond since they are putative descendants of the strain used in the original experiments. That #2038 showed a response and #2036 and #2039 did not may indicate intraspecific genetic variation in susceptibility to EMFs—variation that presumably involves the metabolism of Ca. Any susceptibility may already be lost, however, because of the influence of selection and genetic bottleneck effects on genetic drift (see above). Indeed, with the exception of the experiments on strain III_B (Figs. 10–14), EMF effects were generally ephemeral and the controlling factor (be it genetic or environmental) is, as yet, unknown. Reese *et al.* (1991) also reported an ephemeral response with strain #2038. All these observations further emphasize the importance of using a responsive strain of *A. coffeaeformis* for these experiments, but they also highlight the limitations of *A. coffeaeformis* as a model for assessing EMF interactions with living systems.

We developed the system involving perfusion of media over diatoms as a method for quantitatively monitoring the movements of diatoms exposed to EMFs. The system has

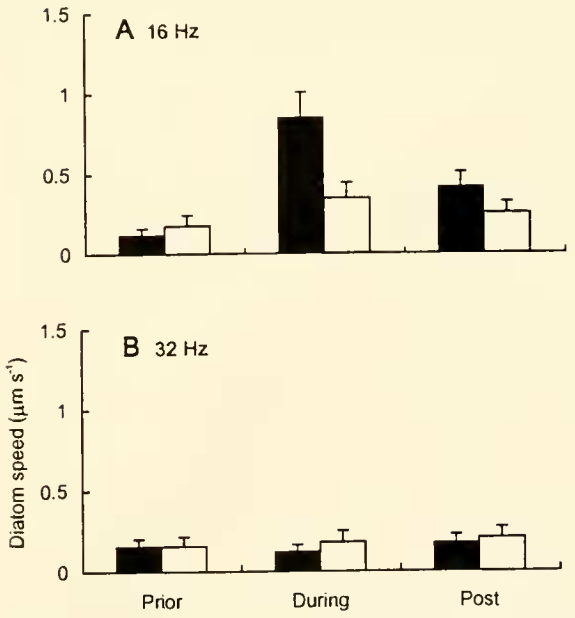

Figure 16. Mean (±SE) speed of 2-day-old #2038 cells in perfusion experiments prior to, during, and after EMF exposure (see text). Filled bars = EMF-exposure; open bars = sham-exposure. EMF before objective lens in place: B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at (A) 16 Hz and (B) 32 Hz, B_V = 0.

several advantages over the agar plate method: it allows real-time *in vivo* recordings to be made of diatom speed and direction while EMF stimulation takes place; diatoms remain alive at the end of the procedure and can be tested again, if necessary; the activities of numerous diatoms can be followed on repeated EMF exposure; positive control tests (motility at high [Ca]; no motility at low [Ca]) can be performed on diatoms at the start and end of each procedure; and the time required for both experimentation and analysis is reduced. Results using this system demonstrated that external [Ca] can be manipulated to limit diatom motility (Fig. 15) and that EMFs at ion cyclotron resonance conditions, but not at an even harmonic (using pre-incubated #2038 cells), increase the speed of motile diatoms (Fig. 16). EMFs therefore act both to increase the proportion of motile diatoms within a population and to increase the speed of those diatoms that show motility.

The positive results reported are consistent with ion cyclotron resonance as the mode of interaction, although tests designed to assess the validity of this mode of interaction were not performed. However, there are theoretical reasons why ion cyclotron resonance is unlikely to be a plausible mechanism (Halle, 1988; Male, 1992). The present results are also consistent with later theories of "ion precession" (Lednev, 1991; Edmonds, 1993), although our experiments give no indication of mechanism, but merely report a possible frequency window. That the Ca^{2+} ion is involved in the process of susceptibility of diatoms to EMFs is not in doubt, and this ion has been implicated in many other studies involving EMF interactions (*e.g.*, Fitzsimmons *et al.*, 1994; Stefano *et al.*, 1994; Gamaley *et al.*, 1995; see Goldberg and Creasey, 1991, and Michaelson, 1985, for reviews). McLeod *et al.* (1992) suggest that biological systems in transition, or those that are working suboptimally, may be most susceptible to EMFs; clearly the diatom system—motility limited by Ca—fits this hypothesis. Nevertheless, the attention paid by workers to the importance of Ca (see Michaelson, 1985) may be unfounded because Ca is a common second messenger in cellular systems (Alberts *et al.*, 1994) and is likely to be involved in the regulation of many cellular processes. Thus the Ca^{2+} ion may not be the link in the biochemical chain that is directly influenced by EMFs, but merely the ion used in destabilizing a system to look for EMF effects and the ion deliberately made rate-limiting. Evidence supporting Ca as the natural rate-limiter in biological processes that can be modulated by EMFs is weak.

In conclusion, we have found the mediation of diatom motility by EMFs to be at best ephemeral and reliant upon the pre-incubation of cells in a specific electromagnetic environment. (Nevertheless, independent replication of bioelectromagnetic phenomena is rare and any such replicability can only help establish bioelectromagnetic science.) In addition, sensitivity to EMFs was demonstrated in only three of five strains of *A. coffeaeformis* tested. It is our opinion that although the "diatom system" may still constitute a useful cellular model for demonstrating bioelectromagnetic effects, further development to identify and control subtle etiological factors is required before the system can be regarded as readily reproducible.

Acknowledgments

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Table I

Effect of light intensity on the motility of diatom strain #2038 exposed for 1 h on 5 mM added Ca plates (n = 3 *in each case*)

Flux density ($\mu E\ s^{-1}\ m^{-2}$)	Diatom motility (%) mean	SE
<0.01	32.33	1.45
0.40	37.00	1.53
9.80	31.33	4.41
23.30	32.00	4.16
53.70	33.00	2.52

Analysis of variance table (performed on arcsine-square root transformed data)

Source of variation	d.f.	SS	MS	*F*	*P*
Light intensity	4	0.00704	0.00176	0.52	0.723
Error	10	0.03377	0.00338		
Total	14	0.04081			

Table II

EMF test results (mean % motilities ± SE on agar plates, n = 3*) at ion cyclotron resonance conditions (B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 16 Hz).* t = t-*value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates*

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Control 5 m*M* Ca
#2036	1.33 ± 0.88	1.33 ± 0.88	0.00	1.00	46
	3.33 ± 0.88	1.33 ± 0.67	1.61	0.25	40
#2038	3.00 ± 1.53	3.67 ± 1.45	0.32	0.77	42
	4.00 ± 1.15	4.67 ± 0.88	0.50	0.65	44
#2039	3.00 ± 1.53	3.00 ± 1.15	0.30	0.79	41
	3.33 ± 0.33	2.33 ± 0.33	2.17	0.12	34
III_B	6.00 ± 1.15	6.00 ± 1.73	0.07	0.95	61
	7.67 ± 0.88	7.33 ± 1.76	0.24	0.83	52
III_F	4.67 ± 1.33	6.33 ± 0.88	1.04	0.41	60
	3.00 ± 1.00	1.33 ± 0.33	1.79	0.22	66

Table III

EMF test results (mean % motilities ± SE on agar plates, n = 3): frequency response. t = t-value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Control 5 m*M* Ca
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 1 Hz					
#2038	7.67 ± 0.88	6.67 ± 0.88	0.79	0.49	46
	4.33 ± 0.88	4.67 ± 1.45	0.10	0.93	42
III_F	6.67 ± 0.33	6.00 ± 1.53	0.51	0.66	56
	3.67 ± 1.20	3.33 ± 0.33	0.14	0.90	52
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 2 Hz					
#2038	7.67 ± 0.88	7.00 ± 1.00	0.50	0.65	49
	8.33 ± 0.33	7.00 ± 0.58	1.98	0.14	51
III_F	2.00 ± 0.58	3.00 ± 1.00	0.72	0.52	58
	1.00 ± 1.00	2.33 ± 0.88	1.38	0.30	54
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 3 Hz					
#2038	2.67 ± 0.88	1.33 ± 0.88	1.14	0.34	44
	2.33 ± 1.20	2.00 ± 1.15	0.12	0.91	49
III_F	5.33 ± 1.33	5.00 ± 0.58	0.16	0.89	56
	4.33 ± 0.33	6.00 ± 0.58	2.57	0.08	50
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 4 Hz					
#2038	4.67 ± 1.33	5.00 ± 1.00	0.25	0.82	46
	1.33 ± 0.88	1.67 ± 0.88	0.19	0.86	48
III_F	4.67 ± 1.76	7.33 ± 0.67	1.45	0.29	50
	10.00 ± 0.58	8.00 ± 1.53	1.27	0.33	52
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 5 Hz					
#2038	1.33 ± 0.33	2.00 ± 0.58	0.97	0.41	46
	0.67 ± 0.33	0.67 ± 0.33	0.00	1.00	47
III_F	2.67 ± 0.67	3.00 ± 1.00	0.24	0.83	50
	4.67 ± 2.19	5.33 ± 1.76	0.28	0.80	51
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 6 Hz					
#2038	4.33 ± 0.88	4.67 ± 1.20	0.19	0.86	39
	2.67 ± 0.33	2.00 ± 0.58	1.03	0.41	46
III_F	7.00 ± 1.00	6.33 ± 0.33	0.61	0.60	56
	4.67 ± 0.33	6.33 ± 0.88	1.82	0.21	58
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 7 Hz					
#2038	3.33 ± 0.88	4.67 ± 1.86	0.42	0.71	47
	5.67 ± 1.20	5.33 ± 0.33	0.19	0.87	42
III_F	7.00 ± 3.06	6.67 ± 2.73	0.04	0.97	56
	5.00 ± 1.53	6.33 ± 1.76	0.54	0.63	48
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 8 Hz					
#2038	1.67 ± 0.88	1.00 ± 1.00	0.60	0.59	36
	6.33 ± 0.33	6.33 ± 0.33	0.00	1.00	38
III_F	1.00 ± 0.58	2.00 ± 1.15	0.46	0.68	49
	4.67 ± 1.86	5.00 ± 1.53	0.21	0.85	50
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 9 Hz					
#2038	5.00 ± 1.00	4.00 ± 1.53	0.63	0.57	46
	0.67 ± 0.67	1.67 ± 1.20	0.71	0.53	49
III_F	5.00 ± 1.53	4.00 ± 1.53	0.45	0.68	59
	1.33 ± 0.88	1.00 ± 1.00	0.43	0.69	43
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 10 Hz					
#2038	5.00 ± 2.08	3.33 ± 2.03	0.75	0.51	47
	0.33 ± 0.33	0.67 ± 0.67	0.24	0.83	41
III_F	2.00 ± 1.53	1.67 ± 0.67	0.23	0.84	51
	2.00 ± 0.58	0.33 ± 0.33	2.66	0.08	49
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 11 Hz					
#2038	1.33 ± 0.33	0.33 ± 0.33	2.23	0.16	42
	0.67 ± 0.67	1.33 ± 0.33	1.35	0.31	36
III_F	7.67 ± 0.88	7.67 ± 1.33	0.04	0.97	43
	6.33 ± 1.76	5.00 ± 1.53	0.54	0.63	39
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 12 Hz					
#2038	7.33 ± 0.88	6.33 ± 1.76	0.58	0.62	42
	2.67 ± 0.33	3.00 ± 0.58	0.45	0.68	38
III_F	8.00 ± 1.15	8.33 ± 2.33	0.03	0.98	51
	7.33 ± 1.76	7.33 ± 0.67	0.10	0.93	56
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 13 Hz					
#2038	0.33 ± 0.33	1.00 ± 0.58	0.88	0.44	44
	1.67 ± 0.88	1.00 ± 1.00	0.60	0.59	44
III_F	8.00 ± 1.00	6.00 ± 1.15	0.06	0.14	56
	6.67 ± 1.45	5.33 ± 1.86	0.63	0.58	60
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 14 Hz					
#2038	3.00 ± 1.53	5.67 ± 1.45	1.33	0.28	49
	6.33 ± 0.33	4.00 ± 2.00	1.08	0.39	53
III_F	6.33 ± 1.45	6.33 ± 1.45	0.00	1.00	48
	0.67 ± 0.67	1.67 ± 1.20	0.71	0.53	51
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 15 Hz					
#2038	1.00 ± 0.58	2.67 ± 1.76	0.59	0.60	48
	0.67 ± 0.67	0.67 ± 0.33	0.34	0.76	57
III_F	3.67 ± 1.20	2.67 ± 0.67	0.71	0.53	39
	4.33 ± 1.20	5.33 ± 2.03	0.33	0.76	46
B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT peak-peak at 17 Hz					
#2038	11.33 ± 0.88	10.00 ± 0.58	1.27	0.29	61
	4.67 ± 1.33	5.00 ± 2.08	0.19	0.19	53
III_F	5.67 ± 0.33	4.67 ± 1.20	0.90	0.47	51
	8.67 ± 0.67	8.33 ± 0.33	0.43	0.71	60

Table III (*Continued*)

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Control 5 m*M* Ca
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 18 Hz					
#2038	5.00 ± 1.15	4.67 ± 1.20	0.21	0.85	38
	0.67 ± 0.67	1.00 ± 0.58	0.53	0.63	37
III_F	3.67 ± 0.88	3.33 ± 1.76	0.50	0.67	71
	7.33 ± 0.88	5.33 ± 2.03	0.99	0.43	64
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 19 Hz					
#2038	0.67 ± 0.67	1.33 ± 0.88	0.64	0.57	41
	1.67 ± 0.82	2.00 ± 2.00	0.23	0.83	43
III_F	9.00 ± 1.73	9.00 ± 2.00	0.01	0.99	42
	5.33 ± 1.20	3.67 ± 2.19	0.85	0.49	40
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 20 Hz					
#2038	4.00 ± 2.65	6.33 ± 0.88	1.04	0.41	46
	0.67 ± 0.67	1.00 ± 0.58	0.53	0.63	36
III_F	1.67 ± 1.67	2.33 ± 1.86	0.39	0.72	50
	1.00 ± 0.58	1.33 ± 0.88	0.16	0.88	42
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 21 Hz					
#2038	1.00 ± 0.58	1.67 ± 0.88	0.36	0.74	40
	2.67 ± 2.67	4.00 ± 2.00	0.55	0.62	36
III_F	10.67 ± 0.88	8.33 ± 1.20	1.53	0.22	61
	6.00 ± 0.58	6.00 ± 0.58	0.00	1.00	54
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 22 Hz					
#2038	2.00 ± 0.58	2.33 ± 1.20	0.20	0.86	34
	1.67 ± 1.67	2.00 ± 1.15	0.41	0.71	41
III_F	5.00 ± 2.08	6.00 ± 1.73	0.45	0.68	56
	8.67 ± 0.33	9.67 ± 1.20	0.78	0.52	59
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 23 Hz					
#2038	3.00 ± 1.53	5.00 ± 1.00	1.19	0.32	46
	5.33 ± 0.33	4.33 ± 1.20	0.85	0.49	40
III_F	3.67 ± 1.67	5.00 ± 1.15	0.77	0.50	72
	8.33 ± 1.20	7.67 ± 0.67	0.44	0.69	59
$B_V = 0\ \mu T$, $B_H = 20.9\ \mu T$, $B_{Hac} = 41.8\ \mu T$ peak-peak at 24 Hz					
#2038	0.67 ± 0.33	0.67 ± 0.67	0.34	0.76	48
	3.00 ± 0.58	2.67 ± 1.20	0.41	0.72	34
III_F	8.00 ± 2.00	7.33 ± 0.67	0.24	0.83	60
	8.33 ± 2.33	6.33 ± 1.20	0.67	0.55	71

Table IV

EMF test results (mean % motilities ± SE on agar plates, n = 3): amplitude response 1. t = t-value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates and exposed and sham-exposed 5 mM added Ca plates

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 5 μT peak-peak at 16 Hz						
#2038	3.67 ± 1.20	3.00 ± 0.58	0.43	0.70	38.00 ± 2.31	43.00 ± 2.08	1.61	0.21
	3.67 ± 2.19	3.67 ± 1.45	0.09	0.93	47.00 ± 1.00	48.00 ± 2.08	0.43	0.71
III_F	10.00 ± 0.58	9.67 ± 1.76	0.25	0.83	67.00 ± 1.00	69.67 ± 1.86	1.27	0.29
	8.33 ± 0.33	8.33 ± 0.88	0.04	0.97	53.00 ± 3.51	57.30 ± 1.86	1.09	0.36
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 10 μT peak-peak at 16 Hz						
#2038	1.00 ± 0.58	1.00 ± 1.00	0.32	0.77	49.33 ± 0.88	47.67 ± 1.20	1.12	0.34
	1.67 ± 1.20	0.67 ± 0.67	0.71	0.53	50.33 ± 0.88	48.33 ± 2.30	0.80	0.51
III_F	3.33 ± 1.45	4.33 ± 20.3	0.32	0.77	61.00 ± 0.58	56.33 ± 1.86	2.41	0.14
	8.67 ± 1.76	9.67 ± 0.88	0.57	0.63	52.67 ± 1.76	51.67 ± 2.19	0.36	0.75
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 15 μT peak-peak at 16 Hz						
#2038	3.33 ± 1.45	3.33 ± 1.45	0.00	1.00	41.00 ± 1.53	46.00 ± 1.15	2.61	0.08
	5.33 ± 0.33	6.33 ± 1.45	0.60	0.61	45.67 ± 2.73	48.00 ± 3.00	0.57	0.61
III_F	7.00 ± 0.58	7.67 ± 0.67	0.76	0.50	61.33 ± 0.88	62.33 ± 1.20	0.67	0.55
	11.30 ± 1.76	10.30 ± 1.33	0.43	0.70	49.67 ± 2.73	56.33 ± 3.18	1.59	0.21
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 20 μT peak-peak at 16 Hz						
#2038	6.33 ± 0.33	6.00 ± 1.73	0.32	0.78	43.00 ± 3.51	47.33 ± 4.10	0.81	0.48
	8.67 ± 0.67	9.67 ± 1.20	0.72	0.52	44.33 ± 2.85	44.00 ± 2.00	0.09	0.93
III_F	6.33 ± 0.33	7.00 ± 0.58	0.99	0.39	55.33 ± 2.60	53.33 ± 3.38	0.47	0.67
	7.67 ± 0.67	7.33 ± 0.33	0.43	0.71	43.67 ± 0.88	42.67 ± 1.76	0.51	0.66
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 25 μT peak-peak at 16 Hz						
#2038	3.33 ± 1.76	4.33 ± 0.33	0.78	0.52	48.33 ± 0.88	43.33 ± 2.40	1.95	0.19
	6.00 ± 0.58	7.67 ± 0.88	1.57	0.21	42.00 ± 1.15	44.00 ± 2.52	0.72	0.55
III_F	2.33 ± 0.88	5.67 ± 1.45	2.00	0.14	62.67 ± 1.76	54.33 ± 3.84	1.97	0.19
	8.00 ± 0.58	8.00 ± 0.58	0.00	1.00	57.67 ± 2.03	54.67 ± 3.18	0.79	0.49
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 30 μT peak-peak at 16 Hz						
#2038	0.33 ± 0.33	1.00 ± 0.58	0.88	0.44	36.33 ± 2.19	38.67 ± 1.45	0.89	0.44
	1.00 ± 1.00	1.00 ± 1.00	0.00	1.00	46.00 ± 2.31	44.33 ± 3.18	0.43	0.70
III_F	9.00 ± 2.52	8.33 ± 0.88	0.17	0.88	53.67 ± 2.33	51.67 ± 0.33	0.85	0.48
	8.67 ± 1.76	9.33 ± 0.67	0.43	0.71	59.67 ± 0.88	57.67 ± 0.88	1.60	0.21
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 40 μT peak-peak at 16 Hz						
#2038	3.33 ± 1.76	2.00 ± 2.00	0.60	0.59	39.00 ± 2.89	44.67 ± 2.40	1.51	0.23
	1.00 ± 0.58	1.67 ± 1.20	0.28	0.80	48.00 ± 2.08	49.00 ± 1.53	0.39	0.72
III_F	7.33 ± 0.88	6.33 ± 2.67	0.52	0.65	51.33 ± 1.86	51.67 ± 2.03	0.12	0.91
	3.33 ± 1.76	1.00 ± 0.58	0.79	0.49	47.33 ± 1.76	49.00 ± 1.73	0.67	0.55
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 50 μT peak-peak at 16 Hz						
#2038	4.33 ± 1.67	3.00 ± 0.58	0.50	0.67	44.67 ± 1.76	46.33 ± 1.86	0.65	0.56
	6.67 ± 1.86	4.33 ± 0.33	1.10	0.39	52.33 ± 2.03	52.33 ± 1.86	0.00	1.00
III_F	6.33 ± 0.33	7.33 ± 0.88	1.06	0.40	50.00 ± 3.46	50.33 ± 3.48	0.07	0.95
	2.67 ± 1.76	3.33 ± 0.33	0.74	0.54	49.67 ± 0.67	51.33 ± 2.40	0.67	0.57

Table IV (*Continued*)

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 60 μT peak-peak at 16 Hz						
#2038	5.67 ± 0.33	7.33 ± 0.88	1.81	0.21	40.00 ± 0.58	42.00 ± 0.58	2.45	0.09
	2.00 ± 0.58	3.00 ± 0.88	1.23	0.31	46.00 ± 1.73	46.33 ± 1.86	0.13	0.91
III_F	5.67 ± 0.33	7.00 ± 1.53	0.74	0.54	57.00 ± 2.00	56.67 ± 1.76	0.13	0.91
	7.33 ± 1.67	5.00 ± 1.53	1.02	0.38	48.67 ± 1.45	51.00 ± 0.58	1.49	0.27
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 70 μT peak-peak at 16 Hz						
#2038	1.00 ± 1.00	0.33 ± 0.33	0.37	0.74	41.67 ± 1.45	46.00 ± 1.53	2.06	0.13
	1.67 ± 0.88	2.00 ± 1.15	0.11	0.92	47.33 ± 1.33	43.67 ± 1.20	2.04	0.13
III_F	6.33 ± 0.33	6.67 ± 0.33	0.71	0.52	61.33 ± 0.88	66.00 ± 2.52	1.75	0.22
	2.00 ± 1.15	3.00 ± 0.58	0.93	0.45	80.33 ± 3.18	83.33 ± 1.76	0.80	0.48
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 80 μT peak-peak at 16 Hz						
#2038	4.00 ± 1.15	3.00 ± 0.58	0.70	0.54	43.00 ± 1.53	45.67 ± 1.67	1.60	0.25
	7.00 ± 1.00	5.00 ± 0.58	1.80	0.17	40.00 ± 0.58	42.67 ± 1.76	1.44	0.29
III_F	0.67 ± 0.67	1.67 ± 1.23	0.71	0.53	57.33 ± 0.88	60.33 ± 0.88	2.40	0.10
	6.00 ± 0.58	7.00 ± 1.00	0.87	0.45	58.33 ± 1.33	61.67 ± 1.20	0.85	0.16
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 100 μT peak-peak at 16 Hz						
#2038	2.00 ± 0.58	4.67 ± 0.88	2.52	0.09	43.00 ± 1.53	43.33 ± 1.33	0.17	0.88
	6.33 ± 1.45	8.33 ± 0.67	1.27	0.33	42.67 ± 2.73	43.00 ± 2.85	0.17	0.88
III_F	2.67 ± 1.20	5.67 ± 1.45	1.62	0.20	72.33 ± 1.76	75.33 ± 2.73	0.94	0.41
	8.67 ± 0.88	6.33 ± 0.33	2.53	0.13	70.00 ± 3.46	75.33 ± 3.18	1.14	0.34
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 120 μT peak-peak at 16 Hz						
#2038	6.33 ± 0.67	4.67 ± 1.20	1.26	0.33	42.33 ± 0.88	44.33 ± 0.33	2.12	0.17
	5.33 ± 1.76	3.67 ± 0.88	0.72	0.52	47.33 ± 0.67	46.33 ± 0.88	0.90	0.43
III_F	8.33 ± 1.20	7.67 ± 0.88	0.41	0.71	73.33 ± 0.88	74.67 ± 2.60	0.51	0.66
	8.67 ± 0.33	8.67 ± 0.33	0.00	1.00	54.00 ± 2.31	51.33 ± 0.88	1.08	0.39
		B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 140 μT peak-peak at 16 Hz						
#2038	0.33 ± 0.33	0.67 ± 0.67	0.24	0.83	46.67 ± 1.20	45.00 ± 1.53	0.86	0.45
	3.33 ± 1.30	3.00 ± 1.73	0.45	0.70	49.33 ± 0.67	50.33 ± 1.20	0.73	0.52
III_F	9.67 ± 0.33	10.67 ± 1.20	0.78	0.51	55.67 ± 2.91	54.67 ± 2.03	0.29	0.79
	7.00 ± 1.15	6.67 ± 1.33	0.21	0.85	48.00 ± 1.53	49.00 ± 0.58	0.61	0.60

Table V

EMF test results (mean % motilities ± SE on agar plates, n = 3): amplitude response 2. t = t-value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates and exposed and sham-exposed 5 mM added Ca plates

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
			B_V = 0 μT, B_H = 2.5 μT, B_{Hac} = 5 μT peak-peak at 16 Hz					
#2038	0.67 ± 0.67	1.67 ± 0.20	0.71	0.53	46.67 ± 0.33	44.67 ± 1.33	1.45	0.28
	3.67 ± 0.67	4.67 ± 0.88	0.88	0.44	46.67 ± 0.67	45.00 ± 1.00	1.39	0.26
III_F	10.67 ± 0.88	10.00 ± 1.53	0.42	0.70	60.00 ± 0.58	60.33 ± 0.88	0.32	0.77
	7.33 ± 0.33	6.67 ± 0.67	0.93	0.45	59.00 ± 0.58	61.33 ± 1.33	1.60	0.25
			B_V = 0 μT, B_H = 5 μT, B_{Hac} = 10 μT peak-peak at 16 Hz					
#2038	2.33 ± 0.33	3.00 ± 0.58	0.98	0.40	47.67 ± 1.45	47.67 ± 1.20	0.00	1.00
	6.33 ± 0.33	6.67 ± 1.20	0.20	0.86	42.33 ± 1.45	41.33 ± 1.20	0.53	0.63
III_F	2.33 ± 0.88	4.00 ± 1.15	1.16	0.33	52.00 ± 1.15	54.67 ± 0.88	1.84	0.16
	8.67 ± 1.20	7.00 ± 0.88	0.47	0.69	54.67 ± 2.40	53.67 ± 2.91	0.26	0.81
			B_V = 0 μT, B_H = 7.5 μT, B_{Hac} = 15 μT peak-peak at 16 Hz					
#2038	0.67 ± 0.67	2.67 ± 0.88	1.98	0.14	38.00 ± 1.15	39.67 ± 1.45	0.90	0.44
	7.67 ± 0.88	6.33 ± 0.33	1.40	0.30	45.33 ± 1.33	45.00 ± 0.58	0.23	0.84
III_F	11.00 ± 1.00	10.67 ± 1.20	0.22	0.84	59.67 ± 0.88	57.00 ± 1.15	1.84	0.16
	7.33 ± 0.33	7.33 ± 0.33	0.00	1.00	48.33 ± 1.20	49.00 ± 0.58	0.50	0.67
			B_V = 0 μT, B_H = 10 μT, B_{Hac} = 20 μT peak-peak at 16 Hz					
#2038	4.00 ± 1.15	4.00 ± 1.00	0.04	0.97	44.67 ± 1.33	46.33 ± 1.76	0.75	0.51
	6.67 ± 1.20	6.00 ± 1.15	0.41	0.71	43.00 ± 1.00	44.33 ± 1.86	0.63	0.57
III_F	2.33 ± 0.88	2.67 ± 0.88	0.25	0.82	45.00 ± 0.00	45.00 ± 0.00	0.00	1.00
	6.00 ± 0.58	8.00 ± 1.00	1.71	0.19	62.33 ± 0.67	62.67 ± 1.76	0.18	0.87
			B_V = 0 μT, B_H = 12.5 μT, B_{Hac} = 25 μT peak-peak at 16 Hz					
#2038	7.33 ± 0.33	7.67 ± 0.67	0.43	0.71	46.00 ± 0.58	46.00 ± 1.00	0.00	1.00
	4.00 ± 1.15	3.33 ± 1.20	0.43	0.69	50.33 ± 1.45	50.33 ± 0.88	0.00	1.00
III_F	10.00 ± 2.00	9.33 ± 1.33	0.22	0.84	49.33 ± 0.88	50.67 ± 2.40	0.52	0.65
	6.33 ± 1.33	7.67 ± 0.88	0.88	0.44	58.67 ± 1.33	57.00 ± 1.73	0.76	0.50
			B_V = 0 μT, B_H = 15 μT, B_{Hac} = 30 μT peak-peak at 16 Hz					
#2038	8.33 ± 0.88	9.33 ± 0.67	0.92	0.43	50.33 ± 1.45	51.00 ± 1.00	0.38	0.73
	4.00 ± 1.00	4.00 ± 1.15	0.04	0.97	43.67 ± 1.86	47.33 ± 1.33	1.60	0.21
III_F	6.00 ± 1.15	6.33 ± 1.33	0.19	0.86	55.33 ± 1.30	56.00 ± 1.53	0.33	0.76
	7.00 ± 0.58	3.33 ± 2.40	1.49	0.27	55.67 ± 0.67	54.33 ± 2.03	0.62	0.60
			B_V = 0 μT, B_H = 20 μT, B_{Hac} = 40 μT peak-peak at 16 Hz					
#2038	7.67 ± 0.67	7.67 ± 0.88	0.02	0.98	51.33 ± 0.67	48.00 ± 1.15	2.50	0.09
	6.33 ± 0.67	4.00 ± 1.53	1.30	0.32	43.00 ± 2.89	43.33 ± 2.96	0.08	0.94
III_F	10.00 ± 1.15	9.33 ± 0.33	0.51	0.66	56.33 ± 0.33	58.00 ± 0.58	2.50	0.09
	11.67 ± 1.20	12.33 ± 1.45	0.35	0.75	52.67 ± 1.86	54.33 ± 1.86	0.64	0.57
			B_V = 0 μT, B_H = 25 μT, B_{Hac} = 50 μT peak-peak at 16 Hz					
#2038	4.67 ± 1.45	5.00 ± 1.73	0.11	0.92	41.00 ± 1.53	40.67 ± 0.67	0.20	0.86
	5.67 ± 0.88	4.33 ± 2.40	0.73	0.54	40.67 ± 0.67	40.67 ± 0.33	0.00	1.00
III_F	7.67 ± 0.88	8.33 ± 0.67	0.61	0.69	53.67 ± 0.88	56.33 ± 1.33	1.67	0.19
	6.00 ± 0.58	7.33 ± 0.33	1.98	0.14	54.67 ± 3.48	57.67 ± 4.10	0.56	0.61

Table V (*Continued*)

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
		$B_V = 0\ \mu T$, $B_H = 30\ \mu T$, $B_{Hac} = 60\ \mu T$ peak-peak at 16 Hz						
#2038	3.00 ± 0.58	3.00 ± 0.58	0.00	1.00	44.67 ± 2.19	44.33 ± 1.76	0.12	0.91
	2.33 ± 0.67	1.67 ± 0.67	0.71	0.52	46.67 ± 0.33	44.00 ± 1.53	1.71	0.23
III_F	3.33 ± 0.67	3.33 ± 0.33	0.08	0.95	56.33 ± 1.45	52.00 ± 1.15	2.33	0.10
	10.00 ± 0.57	8.00 ± 1.15	1.54	0.26	61.00 ± 0.58	63.33 ± 1.76	1.26	0.33
		$B_V = 0\ \mu T$, $B_H = 35\ \mu T$, $B_{Hac} = 70\ \mu T$ peak-peak at 16 Hz						
#2038	2.00 ± 1.15	3.00 ± 0.58	0.93	0.45	46.67 ± 1.20	45.33 ± 1.76	0.63	0.58
	5.33 ± 0.33	5.00 ± 2.00	0.34	0.76	50.00 ± 1.00	50.00 ± 0.58	0.00	1.00
III_F	8.33 ± 0.33	10.00 ± 0.58	2.54	0.09	57.33 ± 1.67	59.00 ± 0.58	0.94	0.44
	10.30 ± 1.20	10.30 ± 0.33	0.05	0.97	52.33 ± 0.67	52.67 ± 0.67	0.35	0.74
		$B_V = 0\ \mu T$, $B_H = 40\ \mu T$, $B_{Hac} = 80\ \mu T$ peak-peak at 16 Hz						
#2038	6.33 ± 0.33	7.33 ± 0.88	1.06	0.40	41.00 ± 1.53	43.67 ± 1.20	1.37	0.26
	4.33 ± 0.67	4.67 ± 2.19	0.03	0.98	41.67 ± 1.86	40.00 ± 1.00	0.79	0.49
III_F	7.67 ± 0.67	7.67 ± 0.67	0.00	1.00	59.33 ± 0.67	60.67 ± 0.88	1.21	0.31
	10.00 ± 0.58	8.67 ± 0.67	1.53	0.22	59.00 ± 0.58	60.67 ± 0.67	1.89	0.16
		$B_V = 0\ \mu T$, $B_H = 50\ \mu T$, $B_{Hac} = 100\ \mu T$ peak-peak at 16 Hz						
#2038	5.67 ± 0.33	6.33 ± 0.33	1.41	0.25	57.00 ± 0.58	54.33 ± 0.88	2.53	0.90
	1.33 ± 0.88	1.00 ± 0.58	0.16	0.88	49.67 ± 1.86	49.33 ± 0.33	0.16	0.89
III_F	10.33 ± 0.88	10.67 ± 1.33	0.18	0.87	59.67 ± 0.33	57.33 ± 2.33	0.99	0.43
	10.00 ± 0.58	9.33 ± 0.88	0.65	0.56	66.33 ± 2.60	67.00 ± 1.53	0.21	0.85
		$B_V = 0\ \mu T$, $B_H = 60\ \mu T$, $B_{Hac} = 120\ \mu T$ peak-peak at 16 Hz						
#2038	1.00 ± 1.00	1.67 ± 1.20	0.52	0.64	39.67 ± 1.20	44.00 ± 1.15	2.60	0.08
	0.67 ± 0.67	1.00 ± 1.00	0.14	0.90	45.33 ± 0.88	51.33 ± 3.38	1.72	0.23
III_F	8.00 ± 1.00	8.33 ± 0.33	0.36	0.76	54.33 ± 0.67	52.67 ± 1.67	0.93	0.45
	11.33 ± 0.67	8.67 ± 1.76	1.44	0.29	49.00 ± 1.00	52.00 ± 2.00	1.34	0.31
		$B_V = 0\ \mu T$, $B_H = 70\ \mu T$, $B_{Hac} = 140\ \mu T$ peak-peak at 16 Hz						
#2038	2.33 ± 0.88	2.67 ± 0.88	0.25	0.82	41.67 ± 3.48	43.67 ± 0.88	0.57	0.63
	0.67 ± 0.33	1.67 ± 0.88	0.61	0.58	42.67 ± 0.67	42.33 ± 1.86	0.17	0.88
III_F	7.33 ± 0.88	7.33 ± 1.67	0.08	0.94	53.33 ± 1.45	55.00 ± 0.58	1.06	0.40
	5.33 ± 0.33	4.00 ± 1.15	1.14	0.37	60.33 ± 0.33	59.00 ± 0.58	2.00	0.14

Table VI

EMF test results (mean % motilities ± SE on agar plates, n = 3): amplitude response 3, t = t-*value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 m*M *added Ca plates and exposed and sham-exposed 5 m*M added Ca plates

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
		B_V = 0 μT, B_H = ambient (~16.5 μT), B_{Hac} = 5 μT peak-peak at 16 Hz						
#2038	1.33 ± 1.33	1.33 ± 0.88	0.29	0.79	37.67 ± 0.88	37.33 ± 1.67	0.18	0.87
	0.67 ± 0.67	0.33 ± 0.33	0.24	0.83	41.00 ± 0.58	43.00 ± 1.15	1.55	0.26
III_F	9.00 ± 0.58	8.67 ± 0.33	0.48	0.66	53.67 ± 2.19	58.33 ± 1.20	1.87	0.16
	6.33 ± 1.45	6.33 ± 0.33	0.10	0.93	61.00 ± 0.58	60.67 ± 0.33	0.50	0.65
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 10 μT peak-peak at 16 Hz						
#2038	3.33 ± 1.76	3.67 ± 1.45	0.38	0.73	38.00 ± 0.58	37.67 ± 0.67	0.38	0.73
	0.33 ± 0.33	0.33 ± 0.33	0.00	1.00	35.33 ± 1.45	33.67 ± 2.19	0.64	0.57
III_F	3.67 ± 0.67	4.33 ± 0.88	0.60	0.59	61.67 ± 0.67	59.33 ± 0.88	2.11	0.13
	3.00 ± 1.15	2.67 ± 0.33	0.09	0.94	54.33 ± 0.67	55.00 ± 0.58	0.76	0.50
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 15 μT peak-peak at 16 Hz						
#2038	1.33 ± 0.33	2.00 ± 1.00	0.54	0.64	39.33 ± 0.88	40.67 ± 1.45	0.78	0.49
	7.00 ± 0.58	7.00 ± 1.00	0.04	0.97	42.33 ± 1.67	42.00 ± 1.00	0.17	0.88
III_F	4.67 ± 0.67	2.33 ± 0.86	2.08	0.13	57.33 ± 0.88	58.67 ± 0.88	1.07	0.36
	4.33 ± 0.88	2.67 ± 0.88	1.33	0.28	58.00 ± 0.58	58.33 ± 0.33	0.50	0.65
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 20 μT peak-peak at 16 Hz						
#2038	2.33 ± 0.88	5.00 ± 0.58	2.39	0.14	45.67 ± 2.03	40.67 ± 2.73	1.47	0.24
	3.67 ± 1.45	3.00 ± 1.53	0.32	0.77	50.33 ± 1.33	49.33 ± 0.67	0.67	0.57
III_F	9.33 ± 0.67	8.33 ± 0.88	0.92	0.43	56.00 ± 0.58	55.67 ± 0.33	0.50	0.65
	7.00 ± 1.00	6.00 ± 0.58	0.82	0.47	52.67 ± 0.88	53.33 ± 1.20	0.45	0.68
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 25 μT peak-peak at 16 Hz						
#2038	4.33 ± 2.03	4.67 ± 2.33	0.07	0.95	42.67 ± 1.45	37.00 ± 2.65	1.87	0.16
	4.67 ± 0.33	4.67 ± 0.33	0.00	1.00	47.00 ± 1.15	46.33 ± 1.33	0.38	0.73
III_F	8.33 ± 1.20	6.33 ± 1.33	1.14	0.34	49.33 ± 0.33	49.67 ± 0.88	0.35	0.76
	11.33 ± 0.67	12.00 ± 1.15	0.48	0.67	53.67 ± 0.88	53.67 ± 0.33	0.00	1.00
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 30 μT peak-peak at 16 Hz						
#2038	4.33 ± 0.33	4.67 ± 0.33	0.71	0.52	45.00 ± 2.00	44.67 ± 1.86	0.12	0.91
	1.00 ± 0.58	1.33 ± 0.88	0.16	0.88	42.00 ± 2.52	42.33 ± 1.20	0.12	0.91
III_F	10.67 ± 1.20	11.33 ± 1.20	0.39	0.72	52.33 ± 0.88	52.00 ± 1.53	0.19	0.86
	9.00 ± 0.58	10.33 ± 1.67	0.68	0.57	59.33 ± 0.67	60.33 ± 0.88	0.91	0.43
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 40 μT peak-peak at 16 Hz						
#2038	0.67 ± 0.67	1.33 ± 1.33	0.24	0.82	40.00 ± 0.58	44.33 ± 2.73	1.55	0.26
	4.00 ± 1.00	5.00 ± 1.00	0.71	0.52	47.33 ± 0.88	47.33 ± 0.33	0.00	1.00
III_F	3.00 ± 1.15	4.00 ± 1.00	0.73	0.52	52.33 ± 0.88	54.00 ± 1.53	0.95	0.41
	9.00 ± 0.58	9.33 ± 0.67	0.37	0.74	57.00 ± 1.00	56.67 ± 0.67	0.28	0.80
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 50 μT peak-peak at 16 Hz						
#2038	3.00 ± 1.15	2.67 ± 0.88	0.17	0.88	39.67 ± 0.67	40.67 ± 0.88	0.90	0.43
	2.33 ± 0.88	2.00 ± 1.15	0.50	0.67	44.67 ± 1.20	45.33 ± 0.88	0.45	0.68
III_F	10.67 ± 0.67	10.00 ± 1.15	0.53	0.67	51.33 ± 1.45	52.67 ± 1.33	0.68	0.55
	7.00 ± 1.15	7.33 ± 0.33	0.34	0.77	47.33 ± 0.88	47.00 ± 1.00	0.25	0.82

Table VI *(Continued)*

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 60 μT peak-peak at 16 Hz						
#2038	2.00 ± 0.58	3.33 ± 1.45	0.74	0.54	44.00 ± 1.15	44.33 ± 0.88	0.23	0.83
	4.67 ± 1.86	3.67 ± 1.20	0.28	0.80	48.00 ± 1.00	48.00 ± 0.58	0.00	1.00
III_F	5.67 ± 0.67	5.33 ± 1.20	0.31	0.79	57.67 ± 1.33	57.00 ± 0.58	0.46	0.69
	7.67 ± 0.67	6.67 ± 0.33	1.36	0.27	51.00 ± 0.57	53.00 ± 1.53	1.23	0.35
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 70 μT peak-peak at 16 Hz						
#2038	2.00 ± 2.00	1.67 ± 1.20	0.18	0.87	42.67 ± 0.67	43.00 ± 0.58	0.38	0.73
	3.00 ± 1.15	2.00 ± 1.53	0.78	0.49	50.33 ± 0.88	49.67 ± 0.88	0.53	0.62
III_F	9.00 ± 0.58	8.67 ± 0.33	0.48	0.66	58.33 ± 1.20	57.00 ± 0.58	1.00	0.42
	9.67 ± 0.33	8.00 ± 0.58	2.45	0.09	52.67 ± 0.88	53.67 ± 0.88	0.80	0.47
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 80 μT peak-peak at 16 Hz						
#2038	0.33 ± 0.33	0.33 ± 0.33	0.00	1.00	41.67 ± 1.20	39.00 ± 0.58	2.01	0.18
	1.67 ± 1.20	0.67 ± 0.67	0.71	0.53	41.00 ± 0.58	40.67 ± 0.67	0.38	0.73
III_F	8.00 ± 0.58	7.33 ± 0.33	0.99	0.39	56.00 ± 1.00	56.00 ± 1.15	0.00	1.00
	10.00 ± 0.58	8.33 ± 0.33	2.54	0.09	56.67 ± 0.33	58.00 ± 0.58	2.00	0.14
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 100 μT peak-peak at 16 Hz						
#2038	2.67 ± 0.88	2.33 ± 1.20	0.48	0.68	37.67 ± 0.88	38.67 ± 0.88	0.80	0.48
	4.33 ± 2.19	0.67 ± 0.67	1.27	0.29	42.67 ± 0.33	41.33 ± 0.88	1.42	0.29
III_F	12.67 ± 0.33	11.00 ± 0.58	2.47	0.09	60.67 ± 0.67	60.00 ± 0.58	0.76	0.50
	12.00 ± 1.15	11.00 ± 1.53	0.55	0.62	48.67 ± 0.88	48.00 ± 0.58	0.63	0.57
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 120 μT peak-peak at 16 Hz						
#2038	1.67 ± 0.67	2.00 ± 0.58	0.43	0.70	33.67 ± 0.88	38.00 ± 1.15	0.46	0.68
	4.67 ± 0.88	3.00 ± 0.58	1.56	0.22	42.33 ± 1.20	42.33 ± 0.88	0.00	1.00
III_F	8.00 ± 1.53	6.33 ± 0.88	0.89	0.44	46.67 ± 0.33	46.67 ± 0.33	0.00	1.00
	7.33 ± 0.67	9.00 ± 0.58	1.86	0.16	59.00 ± 0.58	60.33 ± 0.33	2.00	0.14
		B_V = 0 μT, B_H = ambient (~ 16.5 μT), B_{Hac} = 140 μT peak-peak at 16 Hz						
#2038	1.33 ± 0.88	1.00 ± 1.00	0.43	0.69	39.00 ± 1.15	39.67 ± 1.20	0.40	0.72
	4.67 ± 1.33	3.67 ± 1.45	0.52	0.64	43.33 ± 0.67	42.00 ± 0.58	1.51	0.23
III_F	7.67 ± 0.67	6.67 ± 1.76	0.62	0.60	55.00 ± 1.53	56.00 ± 0.58	0.61	0.60
	8.67 ± 0.33	8.67 ± 0.33	0.00	1.00	57.67 ± 0.67	57.00 ± 0.58	0.76	0.50

Table VII

EMF test results (mean % motilities ± SE on agar plates, n = 3); amplitude response 4, t = t-value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates and exposed and sham-exposed 5 mM added Ca plates

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 5\ \mu T$ peak-peak at 16 Hz					
#2038	1.00 ± 1.00	1.67 ± 1.20	0.52	0.64	35.33 ± 1.76	38.33 ± 0.88	1.52	0.27
	1.67 ± 0.88	2.00 ± 0.58	0.58	0.62	46.00 ± 1.00	43.67 ± 2.73	0.81	0.50
III_F	5.33 ± 0.88	6.33 ± 0.67	0.92	0.42	59.67 ± 0.88	60.33 ± 0.33	0.71	0.55
	7.00 ± 1.00	6.67 ± 1.20	0.24	0.82	54.67 ± 2.03	57.33 ± 0.88	1.21	0.35
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 10\ \mu T$ peak-peak at 16 Hz					
#2038	2.00 ± 0.58	2.00 ± 0.58	0.00	1.00	41.33 ± 0.88	42.33 ± 1.20	0.67	0.55
	3.67 ± 0.33	3.00 ± 0.58	1.02	0.38	47.67 ± 0.88	47.67 ± 0.67	0.00	1.00
III_F	6.33 ± 0.88	8.00 ± 0.58	1.59	0.21	53.33 ± 0.88	52.67 ± 0.67	0.60	0.59
	6.67 ± 0.33	6.67 ± 0.33	0.00	1.00	52.67 ± 1.45	54.67 ± 1.86	0.85	0.46
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 15\ \mu T$ peak-peak at 16 Hz					
#2038	3.00 ± 0.58	3.33 ± 0.88	0.27	0.80	47.33 ± 0.88	47.00 ± 2.00	0.15	0.89
	4.00 ± 0.58	5.66 ± 0.67	1.92	0.15	48.67 ± 0.88	47.33 ± 0.33	1.41	0.29
III_F	2.33 ± 0.88	3.67 ± 0.33	1.45	0.28	52.33 ± 2.03	54.00 ± 0.58	0.79	0.51
	7.33 ± 0.33	6.00 ± 0.58	1.98	0.14	63.33 ± 1.45	63.33 ± 0.67	0.01	1.00
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 20\ \mu T$ peak-peak at 16 Hz					
#2038	2.67 ± 0.33	1.00 ± 1.00	1.78	0.22	48.33 ± 1.20	48.00 ± 1.00	0.21	0.85
	1.00 ± 0.58	0.67 ± 0.67	0.53	0.63	42.67 ± 0.88	41.00 ± 1.15	1.15	0.33
III_F	9.67 ± 0.33	10.33 ± 0.88	0.69	0.56	62.67 ± 1.76	62.67 ± 1.76	0.00	1.00
	8.33 ± 0.33	8.67 ± 0.67	0.43	0.71	58.33 ± 0.88	57.00 ± 0.58	1.26	0.30
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 25\ \mu T$ peak-peak at 16 Hz					
#2038	2.33 ± 0.88	2.67 ± 0.67	0.39	0.72	47.33 ± 0.33	46.00 ± 0.58	2.00	0.14
	4.33 ± 0.88	4.67 ± 1.33	0.11	0.92	46.67 ± 0.67	43.67 ± 1.20	2.18	0.12
III_F	7.33 ± 0.33	7.33 ± 0.88	0.04	0.97	51.33 ± 0.88	53.33 ± 0.33	2.12	0.17
	8.00 ± 0.58	8.33 ± 0.67	0.37	0.74	61.33 ± 0.88	60.67 ± 0.67	0.60	0.59
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 30\ \mu T$ peak-peak at 16 Hz					
#2038	4.33 ± 1.33	4.00 ± 0.58	0.14	0.90	46.67 ± 0.33	45.00 ± 0.58	2.50	0.09
	3.67 ± 0.33	4.33 ± 0.88	0.66	0.58	42.33 ± 0.67	41.67 ± 0.33	0.89	0.47
III_F	10.33 ± 0.33	12.00 ± 0.58	2.53	0.09	59.33 ± 0.67	58.33 ± 0.88	0.90	0.43
	10.00 ± 1.53	11.00 ± 1.00	0.58	0.60	50.00 ± 0.58	53.00 ± 1.15	2.32	0.15
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 40\ \mu T$ peak-peak at 16 Hz					
#2038	3.00 ± 1.53	5.00 ± 1.00	1.19	0.32	46.33 ± 0.33	44.67 ± 1.20	1.34	0.31
	4.00 ± 1.15	3.33 ± 0.67	0.42	0.70	45.67 ± 2.03	47.00 ± 1.00	0.59	0.61
III_F	8.67 ± 0.33	8.33 ± 0.67	0.46	0.69	51.33 ± 0.67	54.00 ± 1.15	2.00	0.14
	6.00 ± 0.58	6.00 ± 0.58	0.00	1.00	52.00 ± 1.00	51.33 ± 1.33	0.40	0.72
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 50\ \mu T$ peak-peak at 16 Hz					
#2038	5.33 ± 1.20	3.67 ± 0.67	1.16	0.33	49.67 ± 0.88	48.00 ± 0.58	1.58	0.21
	4.33 ± 0.33	4.00 ± 0.58	0.54	0.63	40.67 ± 0.88	42.67 ± 0.33	2.12	0.17
III_F	5.33 ± 1.33	5.67 ± 1.20	0.21	0.85	56.00 ± 2.52	56.33 ± 0.88	0.12	0.91
	5.00 ± 1.00	5.67 ± 0.33	0.67	0.57	50.00 ± 1.00	49.33 ± 0.67	0.55	0.62

Table VII *(Continued)*

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Exposed 5 m*M* Ca	Control 5 m*M* Ca	*t*	*P*
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 60\ \mu T$ peak-peak at 16 Hz					
#2038	5.67 ± 0.37	6.33 ± 0.33	0.94	0.45	47.33 ± 0.88	48.00 ± 1.00	0.50	0.65
	6.33 ± 0.33	6.00 ± 0.58	0.52	0.64	43.33 ± 0.88	45.67 ± 1.20	1.57	0.22
III_F	7.67 ± 0.67	8.00 ± 0.58	0.39	0.72	51.33 ± 0.67	53.00 ± 1.15	1.25	0.30
	10.00 ± 0.58	9.33 ± 0.67	0.75	0.51	58.00 ± 0.58	56.33 ± 0.88	1.58	0.21
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 70\ \mu T$ peak-peak at 16 Hz					
#2038	2.67 ± 0.67	3.67 ± 0.33	1.40	0.30	49.00 ± 1.15	50.33 ± 2.40	0.50	0.67
	3.00 ± 0.58	2.67 ± 0.88	0.40	0.72	47.00 ± 1.15	49.00 ± 1.15	1.22	0.31
III_F	7.00 ± 0.58	7.67 ± 0.67	0.76	0.50	61.00 ± 0.58	58.33 ± 1.76	1.44	0.29
	9.67 ± 0.88	9.33 ± 0.67	0.28	0.79	62.33 ± 1.86	60.00 ± 1.15	1.07	0.36
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 80\ \mu T$ peak-peak at 16 Hz					
#2038	1.00 ± 0.58	0.67 ± 0.67	0.53	0.63	43.33 ± 1.45	41.67 ± 0.88	0.98	0.40
	3.00 ± 1.73	5.00 ± 0.58	1.13	0.38	45.67 ± 1.67	49.00 ± 1.73	1.39	0.26
III_F	10.33 ± 0.88	10.67 ± 0.67	0.32	0.77	58.00 ± 0.58	58.67 ± 0.88	0.63	0.57
	8.67 ± 0.33	9.67 ± 1.20	0.78	0.52	53.67 ± 1.76	57.00 ± 1.15	1.68	0.21
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 100\ \mu T$ peak-peak at 16 Hz					
#2038	1.67 ± 0.33	1.33 ± 0.33	0.71	0.52	45.67 ± 2.03	45.33 ± 1.86	0.12	0.91
	2.33 ± 0.88	2.00 ± 0.58	0.25	0.82	41.00 ± 1.15	41.33 ± 1.76	0.15	0.89
III_F	9.33 ± 0.33	10.33 ± 0.33	2.14	0.12	54.33 ± 1.20	53.00 ± 0.58	1.00	0.42
	11.67 ± 0.88	13.00 ± 0.58	1.26	0.30	54.67 ± 1.76	51.00 ± 1.53	1.57	0.21
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 120\ \mu T$ peak-peak at 16 Hz					
#2038	3.33 ± 0.67	2.67 ± 0.33	0.81	0.48	46.33 ± 1.76	46.33 ± 0.67	0.00	1.00
	3.00 ± 0.58	3.33 ± 0.33	0.55	0.62	47.33 ± 1.76	46.67 ± 1.20	0.31	0.78
III_F	7.33 ± 0.33	7.33 ± 0.88	0.04	0.97	57.67 ± 1.33	57.67 ± 1.20	0.00	1.00
	9.33 ± 0.88	9.33 ± 0.33	0.03	0.98	52.00 ± 0.58	50.67 ± 1.20	1.00	0.42
			$B_V = 0\ \mu T$, $B_H = 0\ \mu T$, $B_{Hac} = 140\ \mu T$ peak-peak at 16 Hz					
#2038	2.67 ± 0.33	3.00 ± 0.58	0.45	0.68	47.33 ± 0.88	45.00 ± 1.00	1.75	0.18
	5.67 ± 0.33	4.33 ± 0.88	1.44	0.29	44.00 ± 1.15	45.67 ± 1.20	1.00	0.39
III_F	7.67 ± 0.88	9.00 ± 0.58	1.26	0.30	49.67 ± 1.45	49.33 ± 0.88	0.20	0.86
	7.33 ± 0.88	8.33 ± 0.67	0.92	0.43	51.00 ± 1.00	53.33 ± 1.20	1.49	0.23

Table VIII

EMF test results (mean % motilities ± SE on agar plates, n = 3) at (a) ion cyclotron resonance conditions for the ambient horizontal field at Plymouth, an even harmonic and at 16 Hz; and (b) at switched field axes at ion cyclotron resonance conditions. t = t-*value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates*

Diatom strain	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Control 5 m*M* Ca
(a)					
$B_V = 0\ \mu T$, $B_H = (B_{Hac})/2$ = ambient (16.5 μT) at 16 Hz					
#2038	1.00 ± 0.58	1.67 ± 0.88	0.36	0.74	47
	0.67 ± 0.67	2.00 ± 0.15	0.88	0.44	42
III_F	6.00 ± 0.58	7.00 ± 0.58	1.23	0.31	56
	9.00 ± 1.53	8.33 ± 0.67	0.36	0.75	59
$B_V = 0\ \mu T$, $B_H = (B_{Hac})/2$ = ambient (16.5 μT) at 12.5 Hz					
#2038	1.67 ± 0.67	2.33 ± 0.88	0.60	0.59	46
	1.67 ± 0.33	1.67 ± 1.20	0.46	0.69	49
III_F	8.33 ± 0.33	7.00 ± 0.58	1.98	0.14	59
	6.00 ± 0.58	7.67 ± 0.67	1.91	0.15	61
$B_V = 0\ \mu T$, $B_H = (B_{Hac})/2$ = ambient (16.5 μT) at 6.25 Hz					
#2038	4.67 ± 0.33	5.00 ± 0.58	0.47	0.67	51
	4.00 ± 0.58	3.00 ± 0.58	1.23	0.31	43
III_F	7.00 ± 1.00	6.33 ± 0.33	0.56	0.63	55
	5.33 ± 0.33	5.67 ± 1.33	0.12	0.92	60
(b)					
$B_V = 0\ \mu T$, $B_V = 20.9\ \mu T$; $B_{Vac} = 41.8\ \mu T$ at 16 Hz					
#2038	1.33 ± 0.88	1.00 ± 1.00	0.43	0.69	49
	1.67 ± 1.20	1.67 ± 0.88	0.06	0.96	49
III_F	5.67 ± 0.33	6.67 ± 0.33	2.09	0.13	56
	6.00 ± 0.58	7.00 ± 0.58	1.23	0.31	58

Table IX

Effect of cell density on the motility of diatom strain #2038 exposed at ion cyclotron resonance EMFs for 1 h on 5 mM added Ca plates (n = 2 in each case)

Cell density (cells ml^{-1})	Diatom motility (mean % ± SE)	
	Exposed	Control
5×10^4	40.5 ± 1.5	41.5 ± 1.5
1×10^5	41.5 ± 1.5	43.5 ± 0.5
1.5×10^5	43.0 ± 2.0	40.5 ± 1.5
1×10^6	41.0 ± 3.0	42.5 ± 0.5
2×10^6	45.0 ± 1.0	41.5 ± 0.5

Two-factor analysis of variance table (performed on arcsine-square root transformed data)

Source of variation	d.f.	SS	MS	*F*	*P*
EMF on/off	1	0.000044	0.000044	0.090	>0.25
Cell density	4	0.001202	0.000300	0.612	>0.25
Interaction	4	0.002596	0.000649	1.325	>0.25
Error	10	0.004896	0.000490		
Total	19	0.008738			

Table X

Effect of position in cell cycle on the motility of diatom strain III_F exposed at ion cyclotron resonance for 1 h (mean % motilities ± SE on agar plates. n = 3). t = t-*value of arcsine square-root transformed motility data from exposed and sham-exposed 0.25 mM added Ca plates*

Time of inoculation (h after light commenced at 0700)	Culture age (h)	Exposed 0.25 m*M* Ca	Control 0.25 m*M* Ca	*t*	*P*	Control 5 m*M* Ca
0	101	7.00 ± 0.58	8.00 ± 1.00	0.82	0.47	56
2	99	8.33 ± 0.88	9.00 ± 0.58	0.66	0.56	59
4	97	6.67 ± 0.67	6.33 ± 0.88	0.33	0.76	51
6	95	7.00 ± 1.15	5.67 ± 0.67	0.98	0.40	56
8	93	9.33 ± 0.67	7.67 ± 0.88	1.48	0.23	54
10	91	6.67 ± 0.88	7.67 ± 1.33	0.57	0.61	59
12	89	6.67 ± 1.20	9.33 ± 1.20	1.57	0.21	60
14	87	8.33 ± 1.33	8.33 ± 0.88	0.03	0.98	53
16	85	9.00 ± 0.58	7.67 ± 1.33	0.92	0.45	58

Table XI

Effect of EMFs at frequencies from 1 to 24 Hz (B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT) on the distance moved by diatoms of strain III_F exposed for 1 h on 0.5 mM added Ca plates (distance data pooled from triplicate plates; n = 30 in each case)

EMF frequency (Hz)	Mean distance moved by diatoms ± SE (μm)	
	Exposed	Control
1	46.9 ± 9.3	46.4 ± 10.1
2	28.7 ± 4.3	26.7 ± 4.3
3	33.5 ± 4.6	34.9 ± 5.4
4	25.0 ± 3.2	35.0 ± 5.9
5	35.6 ± 4.4	33.7 ± 4.8
6	38.2 ± 6.9	27.6 ± 3.8
7	34.0 ± 5.4	37.0 ± 7.1
8	37.5 ± 5.6	36.8 ± 7.3
9	35.3 ± 5.7	33.9 ± 5.8
10	37.8 ± 4.8	38.7 ± 6.6
11	46.2 ± 7.8	36.8 ± 6.1
12	35.0 ± 7.8	34.3 ± 6.7
13	38.2 ± 8.4	33.8 ± 5.1
14	35.3 ± 5.3	30.3 ± 4.9
15	35.1 ± 4.7	35.4 ± 6.9
16	36.2 ± 5.5	39.1 ± 6.5
17	37.9 ± 5.1	36.9 ± 6.3
18	35.2 ± 4.1	36.0 ± 6.2
19	41.4 ± 6.3	38.9 ± 6.1
20	32.9 ± 4.3	40.7 ± 5.8
21	33.4 ± 4.5	39.3 ± 5.9
22	39.4 ± 6.8	36.2 ± 5.3
23	37.4 ± 5.1	36.6 ± 5.2
24	38.5 ± 5.0	32.3 ± 4.3

Two-factor analysis of variance table

Source of variation	d.f.	SS	MS	*F*	*P*
EMF on/off	1	424	424	0.40	>0.5
Frequency	23	17930	780	0.74	>0.5
Interaction	23	7490	326	0.31	>0.5
Error	1392	1458958	1048		
Total	1439	1484801			

Table XII

χ^2 values of effect of EMFs at frequencies from 1 to 24 Hz (B_V = 0 μT, B_H = 20.9 μT, B_{Hac} = 41.8 μT) on the initial direction of movement (into one of twelve 30° sectors) of diatoms of strain III_F exposed for 1 h on 0.5 mM added Ca plates (direction data pooled from triplicate plates; n = 300 in each case)

EMF frequency (Hz)	Exposed χ^2 value	*P*	Control χ^2 value	*P*
1	17.83	>0.05	9.43	>0.5
2	10.39	>0.5	15.68	>0.5
3	4.64	>0.9	17.36	>0.05
4	7.28	>0.5	15.92	>0.1
5	6.80	>0.5	12.56	>0.1
6	2.24	>0.99	9.92	>0.5
7	14.96	>0.1	11.84	>0.1
8	2.24	>0.99	5.84	>0.5
9	8.00	>0.5	3.68	>0.9
10	5.12	>0.9	12.32	>0.1
11	14.96	>0.1	3.20	>0.9
12	4.64	>0.9	8.96	>0.5
13	0.80	>0.99	2.00	>0.99
14	0.80	>0.99	9.92	>0.5
15	0.80	>0.99	4.88	>0.9
16	7.52	>0.5	7.76	>0.5
17	8.00	>0.5	5.36	>0.9
18	7.76	>0.5	5.36	>0.9
19	4.16	>0.9	5.84	>0.5
20	3.20	>0.9	1.52	>0.99
21	6.80	>0.5	2.24	>0.99
22	8.48	>0.5	9.20	>0.5
23	4.16	>0.9	7.04	>0.5
24	5.36	>0.9	11.36	>0.1

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